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Wright of Derby

ONE of the greater pleasures of boyhood was the prospect of five or six weeks of holiday completely devoid of thoughts about school. It was in the second or third week that long days of two-a-side football games with the other boys in the street would begin to pall. Simultaneously the semi-annual visit to the dentist loomed large and one bleak morning I would be despatched on the fourpenny-return busride into Derby to be told the results of six months of neglect. The only consolation was spending the rest of the day in the Public Library.

Derby was no delicate maiden of a town, no Salisbury, Ely or Hereford. Instead it had industrial prides such as a large locomotive factory; it built aeroplane engines and, so they said, had the best bus station in the country. This was more relevant stuff to a nine-year-old. The Public Library abutted on to the Museum and Art Gallery and the whole of what might now be called an arts complex, but then was not called anything in particular, was intended to serve a very industrial town.

The library books, among which most of the rest of my post-dental day was spent, were witness to what had made the town great. Shelf after shelf of books on stern industrial subjects, devoted to the self-improvement of the populace. There was a limited number of volumes of a lighter character so after an hour or two of flipping through books explaining conjuring tricks or giving tips on home photography, my attention would wander to the art gallery next door. It was not exactly stacked with crowd-pulling impressionists nor do I recall seeing any Picassos there. It was really a one-man show, and to my mind, a two-painting show. The man was Joseph Wright (1734-97) and the two paintings *The Alchymist*, on our cover this week, and *The Orrery*, on this page.

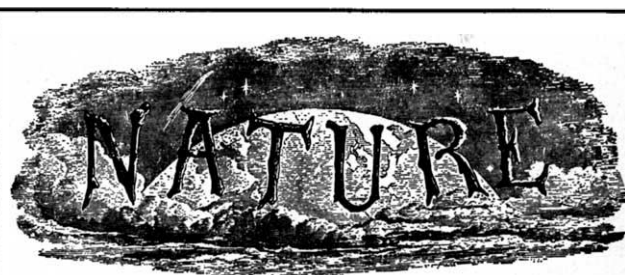
Wright was a contemporary of Reynolds, Gainsborough and Romney and, like them, he made his living from portrait and landscape painting. Unique among painters of that period, or almost any other period for that matter, he was, however, fascinated by the scientific experiments and industrial inventions that were beginning to stir the country's consciousness. He had discovered a rich seam and it is our loss that he did not exploit it more frequently; the *Air Pump* in the Tate Gallery, London is the only other full-scale painting of his on a scientific theme.

Wright used light to the full, particularly the effect of low illumination from candles—indeed it was the sight of *Nature's* staff similarly illuminated which reminded us of the paintings. He was also positively photographic in his detail. The characters are all either local practitioners of science and alchemy or, as the audience, identifiable schoolmasters, businessmen or their children. His observations of scientific instruments seem to have the same precision, and it is reasonable to assume that even the bizarre phosphorescent scene has its basis firmly in actual observation by the artist.

The pictures tell a fascinating story not only of eighteenth century experiments in the name of science but of a time when science was probably more uniformly spread through the country. Each town had its own vigour; the pull of the metropolis and the universities for science barely existed.

The effect of Wright's paintings was always overpowering. The bus journey home to tea was filled with the afterglow of confronting this view of the past. How many other Derby boys had their scientific awakenings from such an unusual source?

100 years ago



WE have received the first Annual Report of the "Haileybury Natural Science Society." It contains preliminary lists of the fauna and flora of the place, together with observations on the meteorology of the locality, and a humorous description of an experimental dinner at which the principal dish consisted of esculent snails which had been specially fed and fattened for the purpose by certain members of the Society. It need scarcely be added, that the repast amply rewarded the members for their generous devotion to the cause of Science.

From *Nature*, 9, 274, February 5, 1874.



Why plant physiology suffers in India

The penurious state of Indian research in plant physiology is described by the President of the International Association for Plant Physiology, Professor H. G. Burström of Lunds University, Sweden.

At a meeting of the Indian Society for Plant Physiology at Simla in 1971 an international committee was set up to examine Indian research in plant physiology*. There has been a general feeling that Indian research in plant physiology is not meeting the demands that could be made on it, and that a better understanding of the subject is now essential for rational exploitation of cultivated plants.

A report was submitted in July 1973¹ which probably reflects conditions in several other developing countries. Many details are of national concern only, but some major problems cannot be solved without international help.

Indian research in plant physiology is mainly done in the botany departments of sixty-five universities (including agricultural universities) and in ten independent research institutions. The committee sent a questionnaire to 150 plant physiologists throughout India, and fifty replies were received from forty-four institutions of different sizes and quality.

About 70% of laboratories report a lack of funds. Restrictions on the availability of foreign exchange make the purchase of chemicals and equipment manufactured abroad difficult and exorbitant prices are demanded. Few chemicals and little equipment are produced in India, and usually when they are, the quality is poor, and often unsuitable for precision work. About half of the laboratories have difficulties in purchasing essential chemicals. Too often scientists have to ask for chemicals as gifts from colleagues abroad. Only about 25% of laboratories have no problems of foreign exchange; these are the institutions maintained by the Government of India.

The University Grants Commission has given financial help to some large universities such as Delhi, Calcutta, and Madras to develop centres of advanced study in certain disciplines of botany, but none so far in plant physiology. In the universities it forms only part of botany, and appropriations go to botany departments. Only a small fraction of the money reaches the plant physiology laboratory, unless the head of the botany department himself is a physiologist. Most of the research in these laboratories is supported by grants for specific projects, amounting to a few thousand rupees a year, quite often not enough to sustain the project itself. Several laboratories have profited considerably from generous grants from the United States PL 480 funds. So there are two extremes—the well equipped and the poorly nourished—and most are poorly nourished.

More than 60% of laboratories consider that lack of instruments limits the choice of research subjects; about two-thirds have difficulty maintaining current research due to lack of equipment—both small and large. Only three or

four laboratories consider themselves well equipped. Foreign industries selling instruments in India provide only sparse maintenance facilities. Few institutions have their own workshops, and instructions on instrumentation and instrument maintenance are hard to come by.

There is thus an inclination towards less expensive research, for example, simple experiments involving only the application of hormones and other chemicals to growing plants. This has serious implications. Although research in more expensive fields is essential due to their application in economic plant husbandry, such studies are rarely undertaken.

Few laboratories have the climate-conditioned growth chambers and glass houses essential for basic research. The ambitious work by Indian physiologists under field conditions lacks reproducibility and the results are liable to several interpretations. In a vast country such as India, with extremes of climate, growth chambers are all the more necessary; although expensive, they save time and manpower. For genuine field trials, however, adequate land space is necessary. Unfortunately even the agricultural research institutions allot to physiological research too small a part of the land available.

In the special physiology units, wherever they exist, staff strength is inadequate. Teams of people with training in analytical methods are usually lacking, and technical assistance is often inadequate. Few scholarships are available for physiological research. In several places there is no provision for research for master's and doctor's degrees in plant physiology.

Library facilities are usually poor. Of the major, exclusively plant physiological journals, *Plant Physiology*, *Physiologia Plantarum*, *Plant and Cell Physiology*, *Journal of Experimental Botany*, and *Planta* are subscribed to by only about 60, 40, 30, 20, and 15% respectively.

In most new institutions back volumes of journals are not available. Even the so-called affluent institutions cannot purchase them for lack of currency. Many institutions do not receive abstracting journals, and the need for an information bank which would supply on request published physiological information is keenly felt. Setting up an information bank, or even strengthening an existing unit, may be expensive, but would be worthwhile.

Many young physiologists complain of the absence of a congenial human environment, lack of encouragement, incentives and appreciation of good work done, lack of drive from superiors, want of opportunities for exchange of ideas, and too many bureaucratic procedures and administrative bottle-necks. The committee has noted cases where the head of a well-equipped laboratory thinks these are no problems, but his subordinates feel otherwise. This human element should not be overlooked, but material deficiencies dominate the picture.

There is a demand from some quarters for the establishment of a Central Institute of Plant Physiology on the pattern of the national laboratories. Probably it will be more useful if five good plant physiology laboratories in north, south, east, west, and central India—one in each zone—are developed into well-equipped Centres of Advanced Study in Plant Physiology, corresponding to the centres set up in other branches of botany. These new centres should also look into the difficulties of the smaller laboratories in the region and help them as far as possible. Workers in adjacent areas should be welcome to work in these laboratories. Association with larger research groups and the organisation of conferences will help the exchange of ideas and partly neutralise the feeling of isolation and frustration expressed by many scientists. Invitations to foreign experts and visits to foreign countries by Indian workers to a larger extent than now will also be useful. This cannot be realised without help from UGC, ICAR, and international funding organisations.

Nevertheless, improvements must begin at the level of the

* Its members were Dr S. Boon-Long, Ministry of Agriculture, Bangkok; Dr R. G. Lincoln, Long Beach, California; Professor S. P. Sen, University of Kalyani, India; and Professor H. G. Burström (chairman).

basic training of students. If only advanced research were encouraged—and this is more attractive than scientific education—a gap may develop between the qualifications of graduated scientists and the requirements necessary for advanced research.

Plant physiological subjects of immediate importance are, for example, mineral nutrition, water balance for irrigation and hardiness problems, hormones as regulators of translocation, growth and development, nitrogen metabolism, and protein production from both old and new sources. Even the highly theoretical topic of photosynthesis has gained in practical importance after the discovery of the special anatomically-linked mode of photosynthesis in tropical and subtropical plant species, responsible for their

high productivity. There is also an urgent need for a study of the physiological parameters of crop yield in the Indian region. Too little or no research is done in these fields in India.

An appreciation of the importance of plant physiological research for crop improvement, and closer collaboration between physiology and genetics with adequate support from the policy-making bodies, will help to improve the situation. But the problem is so vast that international support is needed.

¹ Burström, H. G., Boon-Long, S., Lincoln, R. G., and Sen, S. P., *The present state of plant physiological research in India. A report of its problems* (stencil), 152 pp. Appendix i-iii (Indian Society for Plant Physiology, 1973).

international news

Civil Service scientists take action on pay

John Hall

MEMORIES of the dear dead days of the Aldermaston demos by the Campaign for Nuclear Disarmament (CND) were stirred by the prospect of government scientists broadcasting messages of protest at the gates of their own establishment. An Aldermaston gates meeting was one of the disruptive moves planned as part of a national work to rule started by the scientists' section of the Institution of Professional Civil Servants (IPCS), who are demanding a restoration of salary parity with the Civil Services' administration and executive classes (AEC). The scientists at the Atomic Weapons Research Establishment showed signs of their militant mood as long ago as December, when the announcement of a pay research settlement for the AEC prompted them to fire off a telegram to the civil service department in the following terms:

"Well deserved AEC award rubs salt into wounds already inflicted by CSD. Aldermaston scientists demand justice. Unfair comparisons will bring disruptive action."

A meeting of more than 400 scientists who sent the message decided on "uncooperative" action in January unless a satisfactory pay settlement was made. The mention of unfair comparisons referred to methods of determining what the basis for an increase should be—a problematical question, since, the scientists hold, there are no valid industrial parallels which might help the Pay Board to come to a decision.

The "uncooperative" action was started on January 21, when the IPCS's 20,000 scientific members embarked on a fifteen-point programme of protest actions which included meetings during working hours, half-day strikes and overtime ban. Among the more devious strategies which the disaffected scientists planned was for all suitably qualified staff to apply for every job vacancy advertised in the Ministry of Defence's weekly lists. This would virtually jam the entire machinery of making appointments in key research establishments.

At the end of the first week of 'uncooperative' action at Aldermaston, an IPCS spokesman there declared with satisfaction that scientific work at the establishment had almost come to a stand-still. This was due in large part to the introduction of a scheme requiring all communications within the establishment and with the outside world to be conducted by letter rather than by telephone. No research programme proceeds at white heat when colleagues who are able to wave to each other through their windows elect to exchange views by means of a protracted correspondence.

Industrial action was precipitated by news that the Pay Board, already criticised for being tardy over the scientists' claim, announced that it would not be ready to report before March at the earliest. This news was the straw which broke the camel's back, for it extended the disparity (and the frustration it has generated) that has existed between the pay of scientists and executive civil servants since 1971.

Although there was a correlation between the two branches before that date, a difficulty in assessing criteria for determining a scientist's pay allowed the administrators to jump ahead while the scientists' case was chewed over.

Now, on entry as a Scientific Officer, a graduate can expect to earn £1,300 to £2,200, while his counterpart, the Executive Officer, can expect £1,700 to £2,600. Higher up the scale, the Principal Scientific Officer receives £3,500 to £4,600, while a principal in the administrative group expects £4,100 to £5,400.

In further support of their demands, the scientists point out that between January 1971 and November 1973 the retail price index rose 27.1%, the weekly wage index rose by 39.9%, and the pay of government scientists rose by between 19.4% (in the highest grade) and 23.9% (in the lowest grade). And that included a 7% stage three entitlement.

Nixon announces energy budget

Colin Norman, Washington

In a highly unusual move, President Nixon last week treated the American public to a detailed preview of his Administration's forthcoming budget request for energy research and development in the 1975 fiscal year. The figures, contained in a fact sheet distributed by the White House along with this year's first Presidential energy message to Congress (there were at least three such messages last year), herald a veritable bonanza for research and development aimed at increasing production and use of coal.

The total expenditure proposed for all energy research and development is \$1,810 million, an increase of 81% compared with this year. All areas of research get a look in, but some are more favoured than others—nuclear fission takes pride of place, for example,

carrying off some 40% of the proposed funds, and large increases are earmarked for the development of technologies to control pollution from power plants (a programme whose chief purpose is to enable coal containing high sulphur contamination to be burned to generate electricity).

The spending proposals are the fruit of a frenzied planning process which began in September, when President Nixon asked Dr Dixie Lee Ray, Chairman of the Atomic Energy Commission, to prepare a five-year plan for energy research and development. Her report (see *Nature*, 246, 370; 1973), delivered to the White House early in December, forms the basis of the Administration's budget request, although some of her recommendations have been altered rather drastically (and surprisingly) by the Office of Management and Budget.

Although direct comparisons between Dr Ray's proposals and the budget figures are difficult to make, there are some notable differences in priority. First, the Administration's budget proposals have heaped considerably more money into coal research than Ray recommended—\$577.7 million (including environmental control technology) against \$405 million. Second, Ray's suggestion that \$166.2 million should be spent on developing technology for more efficient use and storage of energy has been reduced; the budget request for such research and development comes out at \$115.7 million. Finally, the total expenditure envisaged by the Administration's budget proposals is a good deal higher than that recommended by Ray—\$1,810 million compared with \$1,570 million.

In line with the goals of President Nixon's much-publicised Project Independence, which are essentially to make the United States self-sufficient in energy as quickly as possible, the spending proposals entail vast concentration of resources in areas which are likely to have a quick payoff, although the reduction in the proposals for conservation research are surprising. Dr Ray in her report that by 1985, conservation efforts are likely to save as

much as 7 million barrels of oil imports per day. She thus recommended that \$1,440 million should be spent on such research and development over the next five years; the Administration's budget proposals recommend only \$700 million during that period of time. Even at that level, however, the outlays next year would be nearly 80% greater than this year's expenditures.

As for longer term efforts, the budget proposals entail expenditures of \$168.6 million on thermonuclear fusion research, a figure which is somewhat misleading since it includes military research on laser fusion. The Administration is in fact suggesting that \$102.3 million should be spent on controlled thermonuclear research, compared with Ray's recommendation of \$135.0 million, and last year's outlay of \$57.0 million.

Very large increases are suggested for solar and geothermal research, however, with the former getting \$50 million and the latter \$44.7 million—increases of 262% and 310% respectively over this year's outlays.

When Dr Ray's report was published in December, it ran into some criticism because it recommended too little money for supporting programmes in basic research, health effects and manpower development. The Administration clearly took heed of such criticisms, for it has added \$216 million to the budgets for such activities next year.

Ray's report also ran into criticism because it paid too little attention to exploiting the technology that should flow from the research programme. In this case, however, the Administration did not respond to the critics, for it reduced by half—to \$50 million—a proposed programme for joint government-industry financing of demonstration plants for coal liquefaction and gasification.

The budget proposals announced last week are far from being money in the bank. For one thing, they must be approved by Congress as part of the usual appropriations procedure (although in election year, members of Congress are unlikely to reduce the requests). For

another, the figures give no details of where the money will come from, or which areas of research (if any) may suffer. That may become clear on February 4, when the rest of the budget will be published.

Boost for coal research in UK

John Hall

IN Britain the energy crisis has wrought a virtual overnight transformation in the role of the National Coal Board (NCB) from caretaker of a sad and declining industry to great white hope for tomorrow's power. Now the board plans to ask the government to finance a £400 million investment programme, which will include an element of more than £25 million for research. The government is not expected to think twice about providing the money.

At the same time, the NCB has advised Lord Carrington, Secretary of State for Energy, to be cautious about committing the country to an expanded nuclear power programme and has asked to meet the Government in order to urge "a change of philosophy".

According to Mr Leslie Grainger, board member for research, Britain has enough coal reserves to maintain current output levels for 100 years or more. The research programme, he said, is directed at making more efficient use of coal than is achieved by current methods of conversion to electricity and the sum requested is realistically costed, as compared with German and United States budgets of £125 million and \$2,900 million.

The NCB is already well advanced in laboratory scale work on the conversion of coal into other forms of energy and expects to have economically competitive plants in operation by the 1980s. The immediate need, therefore, is for hardware and engineering specifications deriving from research experience and pilot plants started within the next year or two could be working productively within the next five years.

A demonstration fluidised combustion boiler generating 20 MW and costing something like £2 million, would probably be the first operational project to emerge from the NCB's new programme. It could be working by 1976 and providing data for a 100 MW plant. A liquefaction plant, scheduled for completion the following year, would produce a fuel, equivalent to a petroleum feedstock, by adding hydrogen to coal dissolved in an oily solvent. An output of 10 tons a day is already a feasible proposition, according to the NCB. At about the same time a start could be made on a pilot gasification unit producing gas

TABLE 1 Federal energy research and development programme (millions of dollars)

Programme area	Program level fiscal year			change 74-75 (per cent)	estimated total FY 1975-79
	1973	1974	1975		
1. Conservation	32.2	65.0	115.7	78	700
2. Oil, gas, shale development	18.7	19.1	41.8	119	400
3. Coal production and utilization	85.1	164.4	426.7	260	2,900
4. Environmental Control	38.4	65.5	178.5	173	800
5. Nuclear fission	406.5	530.5	724.7	37	4,000
6. Nuclear fusion	74.8	101.1	168.6	67	1,600
7. Other	16.5	53.5	154.5	189	900
solar	4.0	13.8	50.0	262	
geothermal	4.4	10.9	44.7	318	
total	672.2	999.1	1810.5	81	

of low calorific value suitable for use with gas turbines. A number of plants might profitably operate on the same site, turning out fuels, chemical feedstocks and energy, and depending mutually on the by-products of each process.

The government's biggest commitment to energy research at the moment is, of course, in the area of nuclear power. In a written reply to a House of Commons question on the amount of investment in research into sources of energy alternative to oil Mr Peter Emery, Under Secretary of State for Energy, said that the United Kingdom Atomic Energy Authority is spending £42.5 million in the current year on research and development of nuclear fission reactors. The major part of this effort is devoted to the prototype fast reactor, which is expected to achieve "a significant level of power output" early this year.

The Central Electricity Generating Board is also carrying out research in the nuclear power field and Britain has an interest in nuclear fission research as an EEC partner. Another European energy venture, is a research programme on hydrogen from water and on solar energy, estimated to have cost the community a total of £0.9 million in 1973.

Spies in Canada's research council

from David Spurgeon, Ottawa

MANY Canadians, including scientists and Members of Parliament, were startled in January by the allegation contained in a television broadcast that their country's National Research Council (NRC) has for many years been serving as a cover for secret intelligence gathering.

Although the NRC unit involved—the Communications Branch—has been in existence since 1940, few Ottawa officials seemed to be aware of its existence or its mission. The director-general of information services in the Department of National Defence—a brigadier-general who has served with the department for some 30 years—told an inquirer that before the television program he had never heard of it.

Those who do know of its mission have been sworn to secrecy. Its director, N. K. O'Neill, refused to give a reporter information on its budget and staff, or indeed to comment on anything concerning it. When asked to whom he reported, he replied that he had been spending all that morning trying to find out. Questioners seeking information from the NRC were directed to the Prime Minister's office (where a spokesman simply quoted from Mr Trudeau's comments on

January 11 in the House of Commons. The Prime Minister acknowledged that Canada "has always collected what information was available to it in its territory", but said it had never to his knowledge "engaged in any espionage abroad in the sense that we have not been looking for information in an undercover way in any other country". And he would not confirm the allegations made in the television program.

The program, aired by the Canadian Broadcasting Corporation (a Crown corporation funded by the federal government), claimed the NRC branch works hand-in-hand with the United States Central Intelligence Agency through the CIA's representative at the United States embassy in Ottawa.

It monitors and records radio traffic received through listening posts in the Arctic and elsewhere, and analyzes this and other intelligence gathered by the Royal Canadian Mounted Police and the armed forces. Encoded messages sent from foreign embassies in the Canadian capital are also monitored. The results of this work are shared with allies such as the United States and Britain.

Regardless of the accuracy of the details, it is apparent that the NRC's Communications Branch is in fact engaged in highly secret work of a nature more usually associated with defence or security agencies than with national research laboratories. Inquiries reveal that the branch came into existence during the second world war, when the NRC seemed the obvious place to go for help because of its scientists' expertise and contacts.

What seems strange is that the work was not moved after the war, either to the Defence Department or the newly-formed Defence Research Board. One reason seems to be that NRC simply provided too good a cover, and since moving the branch would only draw attention to it, those responsible felt it best to leave it where it was. It also seems strange that the matter has never before been raised publicly, because the branch is situated in its own building surrounded by a high wire fence in plain view in southwest Ottawa (albeit far removed from other NRC buildings), and is listed in the government telephone directory.

Now that the branch has surfaced, its work is clearly an embarrassment to the NRC. Those in a position to know say attempts have been made by NRC for years to get rid of it. They also say NRC has contributed nothing to its policy, and that the president and council know little or nothing about it.

The importance of the affair for Canadian scientists is the effect it could have abroad on the image of the country's national laboratories. The NRC has been one of Canada's chief instruments of international science policy and

its representative on many international scientific agencies. To be tarred with the CIA's brush will not help its reputation.

There are national implications too. As Douglas Fisher, former Member of Parliament and newspaper columnist put it in the *Toronto Sun*: "Highly placed people noting Watergate's excesses, get concerned. Could the Communications Branch, NRC, get out of hand? . . . its expenditures and work are never openly questioned or examined".

The questions raised by the television program may, however, lead to changes. David Lewis, leader of the New Democratic Party, which has held the balance of power in the minority government, asked in the Commons: "In view of the fact that the NRC obviously is not the appropriate agency for such an operation . . . may I ask the Prime Minister whether the government would consider taking (it) out . . . and placing it in a more appropriate department. . . ?" To which Mr Trudeau replied that he would consider the suggestion.

Are PWRs good for Britain?

John Hall

WITH the British government hotly tipped to fall, and fall soon, for the attractions of a United States light water nuclear reactor in an attempt to stave off a threatened energy gap, there has been a lot of fast talking in London about the pros and cons of available nuclear options. Both Sir Arnold Weinstock of GEC and Lord Aldington, Chairman of the National Nuclear Corporations, have owned that they favour the American pressurised water reactor (PWR) as the best option for securing cheap power in the 1980s. Their evidence for this preference has not been received as gospel truth, either by the House of Commons Select Committee to which it was presented, or by interested parties which included the Institution of Professional Civil Servants (IPCS) and the Trades Union Congress (TUC).

The energy resources sub-committee of the Select Committee on Science and Technology started hearings on the choice of reactor after information leaked from the Central Electricity Generating Board (CEGB) last autumn to the effect that its chairman, Mr. Arthur Hawkins, had a strong preference for the United States equipment as a quick and cheap answer to Britain's short term power problems. The current programme of advanced gas cooled reactors is at least £500 million over budget, is running late by up to six years and is, according to Mr. Hawkins, a catastrophe which should not be repeated.

The select committee, alarmed at the prospect of a *fait accompli* in this most delicate and expensive area of technological development, immediately concerned itself with an inquiry into the choices before the Government. Its air of scepticism in the face of pro-PWR witnesses like Sir Arnold and Lord Aldington has been undisguised, and when towards the end of January an imminent ministerial decision was rumoured, the committee made an abrupt move (with more witnesses expected to testify) to present its report. That report is expected to say, in effect, that the case for buying American is not proven.

Chief among the factors weighing heavily against the American technology was the questionable safety factor of the PWR, with the spectre of catastrophic pressure vessel failure being raised by government scientists (as part of the IPCS case for buying British) and, less graphically if no less earnestly, by Sir Alan Cottrell, Chief Scientific Adviser to the British Government. The civil servants' man pointed out that a failure of a pressure vessel would let loose on "a trusting populace" a nuclear blast with a force equivalent to that of several Saturn rockets. And Sir Alan, in a memorandum to the select committee, indicated that rupture of the steel vessels involved would not admit the inclusion of any fail-safe device and that the vessels might even be subject to failure caused by corrosion or thermal shock induced by an influx of cold water in a loss-of-coolant accident.

Not that the light water reactors have been unduly maligned on their service records; the suggestion was simply that, although they might be tried and tested on a worldwide scale for smallish systems, insufficient work has gone into safety proving PWRs of the size that the British power system requires. The chief selling points of the PWRs, their cheapness and ready availability, also began to look rather sick when the Nuclear Inspectorate let it be known that it had no details of the proposed designs and that it might take two years to approve the designs when it had them. Such a delay, and the cost of any modifications which the inspectorate might think necessary, take away a few of the attractions of the American system as a short term solution.

A further pro-PWR voice, Sir John Hill, Chairman of the United Kingdom Atomic Energy Authority, hedged his bet by opting for middle term British reactors of the steam generating heavy water variety, with fast breeders following. For immediate needs the American system is probably the best bet, he considered, although he held back at endorsing the CEBG's estimate of a requirement of, initially, 18 LWRs and

ultimately, twice that number. The chairman of the select committee, Mr. Arthur Palmer, made a fairly mild assessment of Sir John's attitude when he said he found him "surprisingly indulgent" to the CEBG's United States line.

The TUC, while reserving its right to make a hard and fast decision, appeared distinctly unnerved by the prospect of imported nuclear hardware and asked for meetings with the CEBG. Apart from expressing concern over the safety (or absence of safety) in PWRs, the TUC's fuel and power committee was said to be worried about the effects of an overseas purchase on the long term development of British nuclear alternatives, and also that the CEBG's estimates of nuclear power requirements would fall short of actual demand. It is bound by a Congress resolution that manufacture and construction for an expanding nuclear energy programme should "wherever possible" be British. The committee has received strong representations from trades unionists that this is one of those occasions on which it is distinctly possible.

Perhaps the strongest protest against the CEBG's plan was made by the IPCS which claims 100,000 members, including 8,000 who work in nuclear energy. Like every other anti-PWR voice in the shouting match. The civil servants were aghast at the safety risks involved in buying a reactor design which was introduced only a year ago and is not scheduled to be translated into an on-line operational plant before 1981. Apparently, unresolved safety problems relating to the effectiveness of the emergency core cooling systems and the continued integrity of the steel pressure vessels over a prolonged period have already caused the American Physical Society to ask for an inquiry.

But the crux of the IPCS case was economic. Its line was that a country with a nightmarish balance of payments problem was not doing itself much good if, at one stroke, it managed to run up a heavy bill in licensing fees for a project which is also calculated to hinder Britain's prospects as an exporter of nuclear power technology. The IPCS reckoned that Lord Aldington's estimate of 100,000 to 200,000 for the first stage licensing fees to Westinghouse was a super-optimistic calculation; it would be more realistic, in its book, to talk rather in terms of millions of pounds for the first one or two reactors.

Its proposal, the IPCS confided to Lord Carrington, Secretary of State for Energy, is to press on with AGRs as the major reactor type used for electrical power in Britain and to build at least one steam generating heavy water reactor (SGHWR) in order to open up

an export market in developing countries.

After the AGRs, the aim should be to move as quickly as possible to HTRs as the basis for the next cycle of power stations, according to the IPCS proposal.

For Britain, the evidence of Sir Alan Cottrell seemed to emerge from the welter of conflicting information as straight talk from an upright citizen with no particular axe to grind. His conclusion about the possibility of the gradual growth of a small crack caused by ageing and corrosion in high stress regions of a PWR might be taken for the general view on the programme as a whole: "it needs further investigation".

Short notes

Continuous microtron

A PROTOTYPE continuous microtron (a cyclic accelerator in which the electrons are accelerated by microwaves) has been constructed in Obninsk, as a result of combined research by a number of institutions throughout the Soviet Union. Until now, it has proved possible to operate microtron accelerators in a pulsed mode only, with a relatively low repetition frequency (typical figures are a pulse duration of 1 μ s and a repetition frequency 100 Hz).

The new continuous microtron is designed to accelerate electrons to energies of 7.5 to 9 MeV with a current beam of up to 2 mA. Thus the power of the beam on the target will be of the order of 15 to 18 kW, considerably greater than with any other type of microtron.

Seismology and power stations

ONE spin-off of the current programme of energy expansion in the Soviet Union has been a survey, carried out by the Academy of Sciences of the Armenian SSR, into seismic activity in the mountains, valleys and ravines of Armenia, with special reference to possible sites for power stations.

The project had two purposes. On the one hand, a team of archaeologists investigated the evidence of earthquake damage in the past; for example, knowledge of the resistance of structures to earthquakes, and the inclination of surviving architectural relics, provides data on the periodicity of earthquakes in the area and the resistance of various local materials to seismic damage. At the same time, a team under Academician A. G. Nazarov studied three reference sites, taking borehole electrical readings to study the vertical profile of the crust.

news and views

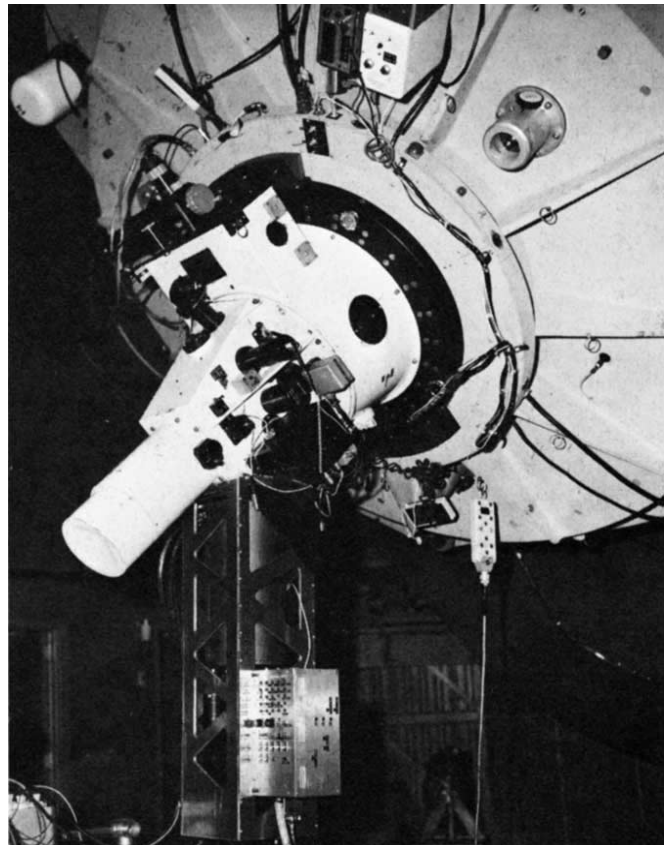
Impact of TV sensors on astronomical research

FOR many decades, optical astronomers have relied largely on photographic materials to record their data. The method is operationally simple, allows large quantities of information to be stored and, perhaps psychologically important, presents those data in a form similar to that perceived directly by the eye. Although the quantum efficiency ($\leq 1\%$) is intrinsically low, values close to those of the best photoemissive surfaces (approximately 20%) can be obtained by using the photographic emulsion in conjunction with an image intensifier tube. The main disadvantages of photographic recording are small dynamic range, non-linear response and difficulty in deriving accurate quantitative data from the plate. These deficiencies become major limitations in, for example, the study of objects which are fainter than the sky and for which background subtraction is therefore necessary. Single channel detectors with the required properties (such as the photo-multiplier tube) have, of course, been used for some time, but recent developments in low light level television-type sensors (see *Proc. Conference on Astronomical Observations with Television Type Sensors* (ed. by J. W. Glaspey, and G. A. H. Walker), Institute of Astronomy and Space Science, University of British Columbia, 1973) have raised the exciting prospect of a linear multi-channel replacement for the photographic work horse. In addition to this primary role of collecting data, television systems are now being used to aid the astronomer in actually operating the telescope. With the aid of a TV monitor, faint objects can be located within a field of stars and placed in the instrument aperture with far greater efficiency than before.

TV sensors come in many shapes and sizes. All, however, have an imaging target which is subsequently scanned by an electron beam. For some tubes, like the silicon target vidicon, the photon-sensitive surface doubles as the storage target. In others, a conventional photo-cathode plus electron imaging optics are used to intensify the image created on the storage target. Most systems currently in use monitor the analog signal output of such tubes. For the range of light levels encountered in astronomy, there have been accompanying problems of linearity, dynamic range, and photometric accuracy. Nonetheless, useful astronomical results ranging from abundance studies of the lunar surface (McCord and Bosel, in Glaspey and Walker, 137) through star cluster photometry (Westphal *ibid.*, 127) to spectroscopy of quasars (Lowrance *et al.*, *Astrophys. J.*, **171**, 233; 1972) have been obtained with these devices. In an attempt to avoid some of the difficulties of the storage analog mode, an image intensifier tube may be placed in front of the TV sensor and the latter then used to count individual photon events (Boksenberg and Burgess; Gilbert; Morton, in Glaspey and Walker, 21, 83, 193). The figure shows one such system operating at the Cassegrain focus of the Steward Observatory 90" telescope. This approach is clearly more accurate and linear but has encountered problems of rather low maximum count rates and of data handling. It thus seems that although TV sensor systems offer great potential in astronomical research, this potential remains largely to be realised. Only in the area of star field acquisition has TV technology so far produced a strong impact on astronomical studies.

It is inevitable that when a new technological advance occurs, wildly enthusiastic claims should be made about its capabilities. In this particular case, one of the wilder claims has been that TV detectors will somehow make large telescopes obsolete. This is nonsense. Increased detective quantum efficiency will increase the effectiveness of large and small telescopes in the same proportion. This gain was made years ago with conventional image tubes and has nothing particularly to do with TV sensors. Certainly for a given observation time smaller telescopes can now do things previously only possible on larger instruments. Far more important, of course, is the fact that large telescopes are able to tackle problems that were not possible before. Even with an ideal detector, many of the important problems of, for example, cosmology are photon limited, especially when competing against the sky background noise. The only way to increase the number of photons is to increase the telescope size whether it be a single large mirror or an array of smaller ones working as a single unit. The latter concept is the basis of the Multiple Mirror Telescope now being constructed by the University of Arizona and the Smithsonian Astrophysical Observatory on Mt Hopkins. The reason for not constructing single mirror telescopes of aperture much in excess of 150" is simply one of cost effectiveness; it has nothing to do with TV sensors.

It should also be remembered that detector technology is advancing very rapidly. At least two systems are already in



A photon-counting TV detector mounted on the Cassegrain spectrograph of the Steward Observatory 90" telescope. The detector package hangs beneath the white spectrograph housing.

operation which, for certain applications, seem more promising than TV sensors. These are the phosphor storage scanner constructed at the Lick Observatory (Robinson and Wampler, *Publs. astr. Soc. Pacif.*, **84**, 497; 1972) and the Digicon pulse counting image tube system developed at the University of California, San Diego (Beaver and McIlwain, *Rev. scient. Instrum.*, **42**, 1321; 1971). Looming on the horizon are self-scanned, semiconductor diode arrays which ultimately promise sensitive reliable imaging in a small package. No doubt all of these systems will seem old-fashioned in three years time. The important thing is for astronomers to take advantage now of instruments available now.

P.S.

G.G.

Behaviour of hybrid primates

ONE of the most interesting questions which faces workers in the area of behaviour of higher animals is the degree to which the behaviour patterns are genetically determined and the degree to which they are determined by the ecological conditions of the species. That has been a particular problem in the study of primates because one of the most important adaptations of these animals is the tremendous flexibility of their behaviour in different circumstances. That has led some people to suggest that there is little or no genetic basis for behavioural differences between species. But the existence of a zone of hybridisation between two closely related primate species in central Ethiopia has provided a unique opportunity for examining the genetic and ecological factors which together determine certain aspects of the behaviour of the two species. These have been the subject of a field study by Nagel, who has now published his findings (*Folia primatol.*, **19**, 104; 1973).

Hybrids between anubis (*Papio anubis*) and hamadryas baboons (*P. hamadryas*) are found at the western end of the Danakil depression (Awash Falls). Although this is not the only hybrid zone to have been studied in primates, the anubis-hamadryas zone is unusual because of the marked differences between both the social systems and the habitats of the two species. The hamadryas baboon (also called the desert baboon) occurs mostly in arid or semi-arid regions in Ethiopia (Kummer, *The Social Organization of the Hamadryas Baboon*, University of Chicago Press, Chicago, 1968). Its social system follows the harem pattern, in which each adult male is permanently associated with one or more adult female. The harems are loosely associated in 'bands' which sometimes forage together; and two or more bands, comprising a 'group', sleep together on one of the sleeping rocks in their desert habitat. Kummer (*Primate Societies*, Aldine, 1971) suggests that the three levels of social organisation of the hamadryas are an adaptation to the extreme conditions of the environment. The smallest social unit, the harem, provides a mechanism for foraging in small groups on the widely dispersed food resources while still guaranteeing that each foraging group has an adult male for protection from predators. The larger aggregations are the means by which the harems can come together to utilise more concentrated resources such as sleeping rocks and water sources.

The anubis baboons have the more common type of social structure, in which there are no permanent associations between males and females and the matings are more or less promiscuous, subject to some bias on the basis of the dominance ranking of the adult males. The anubis baboons are found throughout East Africa and in parts of Ethiopia in savannah habitats. The groups are permanent except for occasional movements of adult males from one group to another, and sleep and forage as a single unit, the members

usually remaining within visual or at least vocal contact with each other.

The hybrid zone is in an arid region which is much more like the usual hamadryas habitat than the usual anubis habitat. It does not coincide with any obvious ecological barrier and in view of evidence for its persistence in more or less the same place for at least the past 60 years, and possibly longer, Nagel has attempted to identify some of the factors responsible for this stability.

Narrow zones of hybridisation between geographic races have been studied in a variety of species, often under the heading of 'area effects'. It is thought that a reduction in the fitness of the hybrid individuals could result in the maintenance of a stable narrow zone. Even in such well studied species as the European land snail (*Cepeae nemoralis* and *C. hortensis*), however, the actual mechanism of the reduction in fitness has not been found. Nagel finds that the reduction in gene flow and the lowering of the fitness of the hybrids can both be accounted for by the differences in the social systems of the two species. Hamadryas males were seen to 'kidnap' older juvenile anubis females in the same way as they would hamadryas juvenile females in order to start their own harems. The abducted females seemed to learn to cooperate with the herding behaviour of the males and fit into the harems, and were seen with hybrid infants. On the other hand, anubis adult males could not mate with hamadryas females because they did not attempt to herd the females and establish permanent bonds with them. Nor could hamadryas males conform to the anubis social system. In consequence, there is a one-way gene flow between the anubis and hamadryas populations, resulting entirely from the absorption of anubis females into the hamadryas harems.

The hybrid zone was identified as the region in which there were harems with hamadryas or hybrid males and anubis, hamadryas or hybrid females. It is important that the hybrid males did not enter the anubis groups: the hybrid males,



according to Nagel, were not as good at herding their females as were the pure hamadryas. This reduction in the fitness of the hybrid males might be sufficient to account for the narrowness of the hybrid zone, for which it can be argued theoretically that only a relatively small reduction in the fitness of the hybrids is needed (Slatkin, *Genetics*, in the press). Because anubis and hybrid males had the same opportunities to learn the herding behaviour as the pure hamadryas, Nagel concludes that the tendency to herd females and form permanent harems is at least in part genetically determined.

Another salient factor, in Nagel's view, is the desirability of small foraging units in the habitat occupied by the hybrid population. Even in the pure anubis group, there was the tendency to break up into foraging groups which would be apart for some part of the day and occasionally sleep apart. The anubis social system was not flexible enough, however, to take full advantage of the small foraging groups and the anubis range was thus limited by the inability to displace the hamadryas from the arid region. This failure to adapt once more suggests a genetic component. On the other hand, the anubis tendency to sleep in trees and the hamadryas tendency to sleep in rocks seemed to have no genetic component, each species apparently utilising the most accessible and the safest (from predators) sleeping sites available.

The existence of the hybrid zone incidentally raises questions about the classification of the genus *Papio*. The situation in the taxonomy of primates is already so complex that the common names of the primates are frequently more useful than the scientific names. Although five species of African baboons have been recognised (*P. hamadryas*, *P. anubis*, *P. cynocephalus*, *P. papio* and *P. ursinus*) there have been observations of free hybridisation between *P. cynocephalus* and *P. anubis* in Kenya (Maples, *Am. J. phys. Anthropol.*, 36; 1972) and now between the anubis and the hamadryas in at least two locations in Ethiopia. The conditions begin to argue for reclassification of *Papio* as a single species or a 'super-species' with different geographical races. Unless emotional attachment to the labelling of fellow primates prevents it, there will have to be yet another change in the nomenclature. Certainly greater differences in morphology of geographic races exist in other species.

M.S.

New method of predicting earthquakes

from our Geomagnetism Correspondent

AFTER many years of pessimism in which limited progress towards a solution

seemed hardly to justify the vast effort of data collection, earthquake prediction studies seem to have entered a new, more hopeful phase. Although it is less than a year since Whitcomb *et al.* (*Science*, 180, 632; 1973) reported their discovery of V_p/V_s ratio changes preceding the San Fernando earthquake of 1971 and proposed a physical model to explain them, Gupta (*Science*, 182, 1129; 1973) has come up with a quite different premonitory phenomenon which involves seismic velocities. As a method of predicting earthquakes it seems to be potentially as useful as the earlier discovery and can be explained in terms of the same physical model, but it has the additional practical advantage that it can be applied using a single three-component seismograph.

The phenomenon itself involves the splitting of S waves and is not, as such, a new discovery. Both laboratory and theoretical studies (see, for example, Nur and Simmons, *J. geophys. Res.*, 74, 6667; 1969) have shown that when non-hydrostatic stress is applied to a rock containing cracks, an anisotropy is induced in the S wave velocity causing the S wave to split into two distinct components with different velocities. By analogy with double refraction behaviour of light waves in stressed glass, the seismic version has come to be known as "acoustic double refraction" and its magnitude, expressed as the difference between the two component velocities, increases as the deviatoric stress increases.

The question now arises, of course, as to whether the phenomenon investigated theoretically and found experimentally in the laboratory can be observed in the field. Gupta has demonstrated that it can, at least in central Nevada where the faulting is primarily related to crustal extension. The main events for which Gupta sought premonitory (and subsequent) S wave splitting were two earthquakes of magnitude 4.0 and 3.9 with epicentres in the Slate Mountain and Mina regions, respectively; and the sources of the observed S waves were the foreshocks and aftershocks of these events. For each main event the difference (Δt) in the arrival times between the two S wave components gradually increased to a maximum and then decreased again towards its original value; and in each case the main shock took place just after the peak Δt had been reached. In numerical terms, the peak values of Δt lay in the range 0.7–0.9 s, which compares with the apparently normal value of about 0.2 s. The peak S wave splitting was thus small (about 2.5% of the total travel time). On the other hand, it was just about the greatest likely to be observed at this location because S wave velocity anisotropy is

greatest along the direction of crustal extension and the source-receiver line was in each case within about 20° of this direction.

On observational grounds alone, therefore, frequent monitoring of Δt values would seem to be a promising prediction technique—and a particularly attractive one in that, in principle, a given area can be monitored using just one three-component seismograph. On a theoretical level, Gupta claims that the observed Δt variation is consistent with pre-earthquake stress accumulation according to the elastic rebound theory, and he has already (*Bull. seism. Soc. Am.*, 63, 1157; 1973) shown it to be explicable in terms of the dilatancy-fluid saturation model used to explain premonitory V_p/V_s changes. The time scales of the two precursors are also apparently comparable. Whitcomb *et al.* found, for example, that the precursor time interval for a magnitude 4.0 event is about 25 days. For Gupta's magnitude 4.0 shock Δt began to rise from its "normal" value of 0.2 s just over 30 days before the shock.

Helical hypodermic

from a Correspondent

BACTERIOPHAGE, literally 'bacteria eater', is the term used to describe viruses which attack bacteria. Some of these are very elaborate, for example, T phage, and have morphologically distinct head and tail assemblies. The phage fastens itself tail first onto the surface of its victim, injects its genetic material through the tail into the interior of the bacterium, and thereby assumes the biochemical machinery of the host and rapidly spawns new phage progeny. The key process of infection is therefore the penetration of the bacterial cell wall by the phage tail. It is powered by a contraction of the phage tail sheath from a long narrow tubular structure to a short fat one. Since the sheath is a relatively simple structure comprising a regular helical arrangement of protein subunits, it provides an excellent system for the study of an elementary contractile process which is not only of intrinsic interest but which may also serve as a useful model in the analysis of contraction in more advanced organisms. In one important respect, however, the sheath's activity differs from that of other contractile systems, such as flagella and muscle, since the contraction occurs once and for all, that is, it is not reversible.

In the case of T-even phages, the presence of sheaths in the extended

and contracted states has often been observed in the electron microscope, and structural studies of the two forms have led to models for the movement of the protein subunits during the contraction. In order to test these models it was necessary to try to follow the process of contraction itself, to catch the phage 'in the act'. In a recent issue of the *Journal of Molecular Biology* (80, 613; 1973) Moody shows how this may be done. From a chance observation he has discovered that treatment with formaldehyde at pH 8 can produce partially contracted sheaths, and from a number of electron micrographs he is able to erect a gallery of views of the sheath at progressively shorter lengths. It is important here to consider whether the chemical treatment produces structures which correspond to those of the living organism, and Moody points out that where partially contracted sheaths can be produced by ultraviolet light (which is generally less effective), the views can be fitted into the same sequence. Thus two different artificial methods produce identical results, which are likely therefore to be substantially the same as in the physiological process. Certainly the formaldehyde causes a smooth and progressive transition into a contracted sheath which appears identical to that normally observed.

The sheath has 144 identical subunits. Both extended and contracted forms show helical lines of subunits, and, in addition, cross striations, which are annuli of subunits. The simplest type of contraction mechanism is one in which all subunits move in the same way (though not necessarily simultaneously) and with this restriction each annulus remains intact during contraction. Different types of subunit movement can in principle be distinguished by the characteristically different appearances of the surface lattice of the partially contracted sheath that they produce. From models elegantly drawn with the aid of a computer and a Calcomp plotter, Moody shows that certain 'transitional' helices are formed as the structure shortens which eventually give rise to a well defined set of helices of short pitch observed in the contracted sheath. Different types of subunit movement produce transitional helices of markedly different appearances, and it is not difficult therefore to correlate the models and micrographs and make the selection. A visual comparison is substantiated by detailed measurements of selected parameters in the micrographs, which show that a smooth transition takes place between one of the prominent helical families in the extended

sheath and the short-pitch helices in the contracted. The subunits do not all move at once. The contraction commences at the base plate and is transmitted by successive annuli, so that a wave of contraction moves towards the head of the phage.

In order to understand more fully how each subunit moves during the contraction, Moody considers the spatial relationships between subunits in the extended and contracted sheaths and shows how the bonding pattern between adjacent subunits may be rearranged during the transition. In regions between subunits where stain and thus solvent is observed to penetrate, there are unlikely to be strong inter-subunit bonds. This reduces the number of parameters that need to be considered. During contraction the bonds between the units of each annulus may undergo only minor realignments (of the type, for example, observed in haemoglobin during the oxy/deoxy transition), but larger movements are required along the 'transitional' helices. The larger movements must involve sliding of the subunits over each other and probably also changes of the subunit shape.

Large movements are permissible in an irreversible process like the sheath contraction, in contrast with the small movements that are a prerequisite of the reversible processes involved in other contractile systems. Moody proposes that the extended form of the sheath is unstable. The formation of this unstable configuration is either dictated by the structure of the base plate, which forms a template for the sheath assembly, or it may be stabilised by the core, which has the same helical symmetry. In either case, it apparently requires only a small stimulus for the stored energy of the sheath to be released. Since the stimulus, arising at the base plate, is transmitted by the wave of contraction itself, the sheath has the properties of both excitation and contraction, and may be considered a primitive 'nerve-muscle'.

Vole aggression and population density

from our *Animal Ecology Correspondent*

AMONG the theories purportedly governing rodent populations, Chitty proposed one to explain the return to 'normal' densities from 'high' densities during cycles of population (*Proc. ecol. Soc. Aust.*, 2, 51; 1967). The theory held that the return is brought about by a balanced polymorphism of aggres-

sive behaviour phenotypes shifting in favour of enhanced aggression when population increases. This phenotype adversely affects reproduction and juvenile survival. Population decline is the inevitable result.

There is nothing basically different between a 3 or 4 year cyclical pattern of increase and decrease and the normal annual pattern. The same sort of pressures are put on the same resources, so it would be logical to assume that the mechanisms which return population density from high to low are, in each pattern, the same. That assumption calls for a demonstration of the existence of an annual cycle of aggressiveness. This has now been done by Turner and Iverson working with *Microtus pennsylvanicus* (*Ecology*, 54, 967; 1973), and their study joins those of Sadlier (*J. Anim. Ecol.*, 34, 331; 1965), Healey (*Ecology*, 48, 377; 1967) and Krebs (*Ecology*, 51, 34; 1970) as evidence supporting Chitty's theory.

Turner and Iverson's study was a mixture of field and laboratory observations conducted on a population of *M. pennsylvanicus* occupying an old field habitat at Pinawa, Manitoba. Demographic data were collected from a trapping grid during fortnightly trapping periods each of 2-4 nights' duration. The study lasted 17 months. The level of aggression in the population was determined by observing, for 10 min periods, the number of 'aggressive acts' performed in an arena by two male combatants taken from a representative sample of the wild population. The main acts of aggression used in the analysis were 'threat' (aggressive lunge of one vole towards another), 'vocalisation' (squeaking audible to the observer), 'mutual upright' (violent bipedal boxing) and 'fighting'.

Several attempts have been made in the past to convey a comprehensive picture of aggression. Sadlier and Healey, working with *Peromyscus*, used a sum of six aggressive acts and the total number of threats and chases respectively. Krebs used the number of attacks per approach in *M. ochrogaster* and *M. pennsylvanicus*. Turner and Iverson are the first workers to regard vocalisation as an aggressive act. Vocalisations occur very frequently and any straightforward numerical analysis would over-emphasise their importance. They weighted the four aggressive acts equally. These were then subjected to principal component analysis, and an index of aggression for each encounter was produced by multiplying the weighted values of each act by the factor determined by the principal component analysis. The index showed that aggression increased in frequency at the onset of reproduction and decreased

with cessation of breeding. The increases and decreases were statistically significant.

When various population parameters were examined it was found that home ranges in the breeding season were significantly larger than those in the non-breeding season. Home ranges of overwintered voles in the breeding season were significantly larger than those of young born in the breeding period. Perhaps surprisingly, there was no significant relationship between an individual's level of aggression and its home range size. Adult voles were more aggressive than young and resident home range holders were more aggressive than non-residents.

All the data collected point to the complicity of rapid change in aggression at the time of most rapid change in population size. Eventual intrinsic control of population could be caused either by a strict spacing behaviour or by an interference with breeding performance in the manner suggested by Chitty. Either way, aggression could be seen to play an important part.

Lidiker's long term work on an island population of *M. californicus* (*Ecol. Monogr.*, **43**, 271; 1973), reported in these columns a few weeks ago (see *Nature*, **246**, 380; 1973), suggested an alternative type of natural control in which no one factor was markedly more important than several others. Quite obviously much more detailed study of the complex processes of rodent population stability is necessary if the answer (if, indeed, there is to be just one answer to a problem which occurs wherever rodents exist) is to be found. One thing is clear. Dynamic stability, or the lack of it, is the result of behavioural and physiological change, not the cause of it.

High spin states of nuclei

from our Nuclear Theory Correspondent

REACTIONS between heavy ions are proving valuable tools for the investigation of high spin states of nuclei at high excitation energies. Heavy ions readily produce such states because they bring energy to the compound nucleus without high velocities; for example, 100 MeV can be brought in by a ^{10}B nucleus or by a single nucleon but the velocity of the nucleon would be much higher than that of the ion and so there would be a greater likelihood of direct reactions or of disintegration of the target nucleus. Furthermore, if the collision takes place non-centrally the compound nucleus is given a large angular momentum, so

there is an enhanced probability of exciting high spin states.

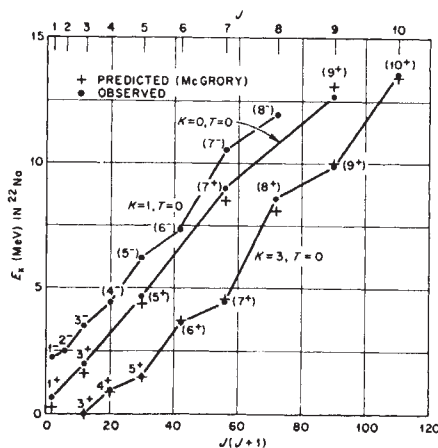
This selectivity of heavy ion reactions is important because at moderately high excitation energies there is often a high density of nuclear states. Only a very few of these have high spins and a heavy ion measurement at relatively low resolution is able to pick them out easily whereas a less selective reaction would require much higher resolution to show them with the same degree of clarity.

The spin of a particular state may be obtained by comparing the angular distribution of the reaction leading to it with the predictions of the statistical theory of nuclear reactions due to Hauser and Feshbach. This theory predicts that the angular distribution is symmetric about 90° in the centre-of-mass system and gives cross sections that depend quite critically in shape and magnitude on the assumed spin of the final state. The identification of the reaction mechanism may be further checked by studying the characteristic fluctuations in intensity of the emerging particles as a function of incident energy.

This method of studying high spin states has recently been used by Gomez del Campo, Ford, Robinson, Stelson, McGrory and Thornton (*Phys. Lett.*, **46B**, 180; 1973) to determine the energies and spins of rotational states in ^{22}Na by the $^{10}\text{B}(^{16}\text{O}, \alpha)^{22}\text{Na}$ reaction.

The angular distributions of the alpha particles emitted at energies corresponding to several states of ^{22}Na were found to be very well fitted by the Hauser-Feshbach statistical model calculations, confirming both the assigned spins and the assumed character of the reaction.

These high spin states in ^{22}Na are



Excitation energies of states in three rotational bands in ^{22}Na as a function of $J(J+1)$. The dots refer to the measured energies and the crosses to shell model calculations.

members of rotational bands and the dependence of their energies on $J(J+1)$ is shown in the figure. It is clear that the bands can be followed to high spin values, and that there are systematic deviations from the $J(J+1)$ proportionality. Further confirmation of the spin assignments is provided by the energies given by shell-model calculation, and these are also included in the figure.

This example shows the advantages of heavy ion reactions for studying high spin states, and it is likely that this method will be extensively used in the future.

Surface changes in transformed cells

from a Correspondent

THE idea that the composition of the cell surface controls to some extent the orderly proliferation of cells in culture now occupies a central position in cell biology. The classical experiments of Aub and his colleagues and later of Burger and Sachs and their collaborators demonstrated surface changes in a particularly simple manner by agglutination with certain lectins. The susceptibility of cells to agglutination could be correlated with the growth characteristics of these cells in culture. Subsequent arguments concerning the basic mechanisms behind the increased agglutination of transformed cells compared with non-transformed cells as induced by concanavalin A or wheat germ agglutinin have left the problem still not completely resolved. But there is general agreement that the changes are essentially small quantitative differences in the amount of receptors at the cell surface able to form attachments with multivalent lectin ligands, or in the topographical distribution of these sites.

Clearly, receptors for wheat germ agglutinin, for instance, are present on normal as well as transformed cells and some progress has been made in their characterisation from both cell types (Jansons and Burger, *Biochim. biophys. Acta*, **291**, 127; 1973). The demonstration of clear qualitative change in the surface membrane composition of cells that can be correlated to growth control would therefore be important. What may turn out to be just such a change in cells and their virus induced transformants is described by Hynes (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3170; 1973).

What Hynes has done is to treat clones of the hamster fibroblast line NIL, which show normal behaviour in culture, with lactoperoxidase. In this process external proteins on the surface membrane of the cells are iodinated with

^{125}I and can be identified by radioautography after separation of membrane components by SDS-polyacrylamide gel electrophoresis. Several iodinated proteins are identified in the normal NIL cells. If cells pretreated with lactoperoxidase are subsequently exposed briefly to a low concentration of trypsin, all but one (of molecular weight about 130,000) of the iodinated proteins bands disappear from the radioautograph of the polyacrylamide gel. Clearly therefore these components have been cleaved from the cell surface, whereas one iodinated protein is resistant to proteolysis.

When virally transformed cells were examined a different result was obtained. In these cells an iodinated band of molecular weight greater than about 200,000 is missing from the radioautographs but is clearly visible in radioautographs prepared from normal cells. The radioautographs are otherwise identical. In some cases, polyoma-transformed cells for instance, there is not a complete loss of the high (greater than 200,000) molecular weight band, but in all cases a substantial reduction is seen in the amount of iodinated material in this region of the gel. Hynes further shows that the band in normal cells is particularly sensitive to proteolysis of intact cells and can be removed from the surface of normal iodinated cells significantly faster than the other bands that were progressively degraded during more extensive proteolysis.

The same experiments were repeated with a representative collection of normal cell lines (hamster, mouse, chick) and their viral transformants (hamster sarcoma, polyoma, RSV) with essentially the same results. Furthermore, the differences observed could not be related to the state of growth of the cells since confluent quiescent cells and non-confluent rapidly dividing cells gave identical iodination patterns.

The conclusions from these experiments clearly mean that a protein, apparently a glycoprotein, is available to lactoperoxidase in many normal cell lines but is not iodinated after transformation. Either the glycoprotein is missing entirely from the cell surface of transformed cells and presumably is not synthesised by these cells, or the glycoprotein is present on the surface membrane but is not accessible for iodination. It will be recalled that this second explanation was originally used for the decreased agglutination of normal cells by concanavalin A or wheat germ agglutinin. Subsequent attempts to demonstrate differences in binding of labelled lectins by normal and transformed cells have, however, in general been unconvincing. In the best case an increase of only 3-5 fold in transformed cells is claimed (Noonan and Burger, *J. biol.*

Chem., **248**, 4286; 1973). Clearly the increased labelling of the high molecular weight band by lactoperoxidase treatment of normal cells compared with transformed cells is considerably more impressive than these values.

There is the intriguing possibility that the iodinateable band is present transiently in transformed cell membranes but is released by a high level of endogenous protease. The amount of proteolytic digestion that removes the band from iodinated normal cells is about the same as that which causes normal cells to grow to high cell densities, that is to escape temporarily growth control and act as transformed cells. Whether or not these events are mere coincidences remains to be established. It is tempting, however, to infer a causal relationship between a high level of endogenous proteolytic activity in transformed cells, loss of the high molecular weight surface glycoprotein and insensitivity to contact inhibition of growth. It will now be of great interest to characterise further the glycoprotein and to establish what function if any it has in the events of contact inhibition and growth control. Its ready release from cells by mild proteolysis may very well contribute to this end.

Sediments in archaeology

from a Correspondent

AN interdisciplinary discussion on sedimentological analysis in archaeology was organised by D. A. Davidson (St David's University College, Lampeter) and M. L. Shackley (University of Southampton), at the University of Southampton on December 15-16. Participants were drawn from a wide range of disciplines—archaeology, pedology, geography, geomorphology, geology and the biological sciences. The meeting was financially supported by the British Academy and the Royal Society.

Attention was first directed towards methods of field recording of archaeological sediments and their subsequent laboratory analysis. Controversy became apparent over the longterm usefulness of sediment recording systems. On the one hand there is need to record as much information as possible on an archaeological site but on the other there is always the danger that the recorded data may become obsolete with the development of new problems and techniques. J. Catt and A. Weir (Rothamsted Experimental Station), in a review of laboratory techniques, stressed the need for selectivity in sample size and analytical technique be-

cause most methods are very time consuming. The use of petrographic, heavy mineral and are spectrographic techniques in assessing the provenance of sediments used in ceramics was demonstrated by J. LL. W. Williams and D. A. Jenkins (University College of North Wales, Bangor). Consideration of specific techniques was followed by discussion of coastal palaeoenvironments and the chronology of sea level change. C. Delano-Smith (University of Nottingham), on behalf of I. Morrison (University of Edinburgh), discussed an analysis of more than 1,000 radio-carbon-dated strata directly involved in marine changes along the western seaboard of Europe during the Holocene. It was suggested that eustatic change during this period has been marked by short term oscillations rather than by gradual changes.

Much of the meeting was devoted to terrestrial sedimentary environments with specific reference to human occupation. A. V. T. Kirkby and M. J. Kirkby (University of Leeds) have established an association between form and age of house mounds at sites in the Oaxaca Valley, Mexico. Consideration of site burial by alluviation also suggests that surface evidence by a scatter of sherds is quickly lost. A. Straw (University of Exeter) by describing a site at Welton-le-Wold in Lincolnshire presented a neat example of the use of geomorphological and sedimentological techniques in the reconstruction of a Pleistocene environment. A similar approach was adopted by A. J. Tankard and F. R. Schweitzer (University of Cape Town) in their analysis of the intricate cave sediments at die Kelders, Cape Province; parameters of grain size provide clues to former sedimentary environments within the cave. The relations between distribution of sites and spatial variation in sediment was also considered; for example, J. L. Bintliff (University of Cambridge) examined pre-Roman settlement patterns in selected parts of southern Greece and illustrated a spatial relation between sites and fertile soils. A sedimentological approach to settlement patterns was also adopted by C. Delano-Smith for the Tavoliere of Foggia, Italy.

The final part of the meeting was devoted to the biological characteristics of sediments. The results of pollen analysis have been widely utilised in archaeology and G. W. Dimbleby (Institute of Archaeology, London) summarised problems in the interpretation of palynological evidence. Contributions by P. Buckland (Doncaster) on insect remains and J. Evans (University College, Cardiff) on molluscan faunas concluded the meeting. The proceedings will be published in 1975 by Duckworth.

Control of Cell Division by Very Lysine Rich Histone (F1) Phosphorylation

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F1 histone phosphorylating activity correlates closely in the cell cycle with the behaviour of the mitotic trigger. It is proposed here that the phosphorylation of F1 histone is the initiation step for mitosis.

THE control of mitotic cell division, a universal and fundamental aspect of all eukaryote life, is the basis of the cancer problem. The control process involves an interaction between the nucleus and the cytoplasm and here we present a proposal for two steps, at the molecular level, in this interaction leading to mitosis. The proposal involves phosphorylation of F1 histone, which is controlled, presumably from the cytoplasm, by a nuclear F1 histone phosphorylating activity. We present here detailed experimental data in support of the proposal and suggest methods for further testing.

Histones have been implicated in most chromosome functions by various authors although it now seems unlikely that functions, such as specific repression, which require a wide range of tissue and species specificity can be carried out by histones. Indeed, the remarkable conservation of the F2A1 histone primary structure during evolution¹ argues strongly that this histone, at least, performs a function that is exactly the same in all eukaryotes and that involves a very precise molecular interaction. One probable function of the four histones F2A1, F2A2, F2B and F3 is the maintenance of the normal interphase tertiary structure of DNA. It is clear that the aspects of this structure observed in calf thymus chromatin by X-ray diffraction are not caused by F1 histones².

Post-synthetic covalent modifications of histones have been observed and may be important in the function(s) of these histones. In one case, the phosphorylation of F1 histone, the modification is reversible and occurs in a cyclic fashion^{3,4} with both old and newly synthesised histone being phosphorylated⁵. We have shown that the maximum phosphate content of the very lysine rich histone in *Physarum polycephalum* (Pp F1) occurs in late G2 phase at exactly the time that chromosome condensation before mitosis becomes visible in the phase contrast microscope (± 0.005 of a generation time). Data from mammalian systems are fully consistent with these observations. This and other experiments led to our proposal that phosphorylation of lysine rich histone causes chromosome condensation⁶.

To study the control of Pp F1 phosphorylation, chromosome condensation and mitosis, it is important to know if the phosphorylation is caused by a change in enzyme activity or by a change in substrate availability. Substrate availability could be effected by a change in chromatin structure and, if this were found to be the controlling factor, Pp F1 phosphorylation might be a trivial consequence of chromosome condensation. On the other hand, a change in the enzyme activity would strongly imply that Pp F1 phosphorylation is a necessary preliminary step in chromosome condensation. Changes in histone phosphate content can be achieved by the action of a

histone phosphokinase or a histone phosphatase⁶. The factor controlling histone phosphate content is the net histone phosphorylating activity in the nucleus. Here we report the results of a series of measurements of the net phosphorylating activity of nuclei of *P. polycephalum*, isolated at defined stages of the mitotic cycle, acting on added calf thymus F1 histone.

P. polycephalum is a true slime mould. In the plasmodial stage the nuclei go through mitosis synchronously and such synchronous divisions can be maintained for at least five mitoses. The nuclei behave like typical eukaryote nuclei except that the nuclear membrane remains intact during most, if not all, of mitosis. The mitotic cycle time of our specimens is 8 to 9 h; G1 phase, if it exists at all, is less than 10 min; S phase is about 3 h and G2 phase is about 5 h. *P. polycephalum* histones resemble calf thymus histones^{3,7}. It is assumed here that Pp F1 has the same function and mode of action as calf F1 histone, as the amino acid compositions are very similar, although it is known that Pp F1 moves slower during polyacrylamide gel electrophoresis^{8,7}.

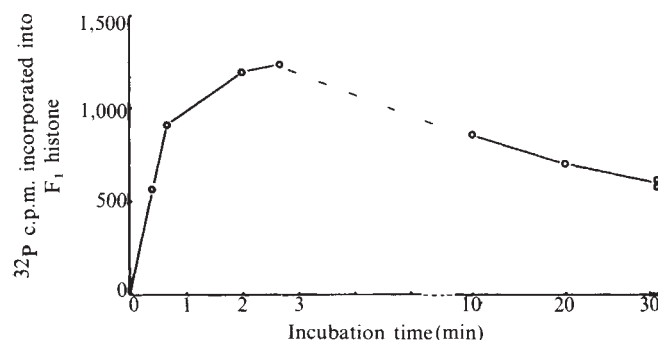


Fig. 1 Histone phosphorylating activity in *P. polycephalum* nuclei. Microplasmodia of *P. polycephalum* were grown in shake flasks in a semi-defined medium⁴². Nuclei were isolated by a modification of the method of Mohberg and Rusch⁴³ in which $MgCl_2$ was substituted for $CaCl_2$, the sucrose concentration was increased from 1.0 M to 1.6 M and a Polytron sonicator was used instead of a Waring blender. The nuclear pellet was washed twice with 10 mM $MgCl_2$ +10 mM Tris-HCl, pH 7.2. Each incubation mixture contained 50 μ mol Tris-HCl, pH 7.2, +5 μ mol $MgCl_2$ +1.5 nmol cyclic 3',5'-AMP+0.5 μ mol theophylline+1 mg calf F1 histone+ 10^8 nuclei in a final volume of 1.5 ml. The reaction was started by the addition of 1 μ mol γ -³²P-ATP (1 to 2 Ci mmol⁻¹). After incubation at 25°C the reaction was stopped by the addition of an equal volume of 10% trichloroacetic acid (TCA) containing 5 mM sodium pyrophosphate and 1 mM sodium phosphate¹⁰. After centrifugation of the assay mixture at 2,000g for 20 min, the pellet was extracted with 1 ml 0.2 M $CaCl_2$ at 80°C for 5 min. The mixture was centrifuged as before and the two supernatants pooled. 1 mg calf F1 histone was added as carrier and TCA was added to a final concentration of 25%. The histone was purified by four washes in 25% TCA and extraction of the precipitate with 0.02 N H_2SO_4 by dialysis overnight at 4°C. The extract was centrifuged at 20,000g for 30 min and the supernatant radioactivity determined by Čerenkov counting in a liquid scintillation counter⁴⁴.

Nuclear histone phosphorylating activity

P. polycephalum nuclei were isolated, sonicated and incubated in the presence of γ - ^{32}P -ATP, calf F1 and cyclic 3'5'-AMP as described in the figure captions. ^{32}P incorporation into calf F1 was demonstrated by acrylamide gel electrophoresis of the radioactive product. All the radioactivity migrated with the calf F1 band, located by staining (R. J. I., PhD thesis, in preparation). The time course of incorporation was somewhat unusual. With the lower ratio ATP: nucleus the incorporation occurred in the first 2 min followed by a slower net removal of ^{32}P from the substrate (Fig. 1). These kinetics are tentatively interpreted as being due to the combined action of a phosphokinase activity and a phosphatase activity⁸. Initially, the ^{32}P ATP concentration is high and the ^{32}P calf F1 specific activity is zero, so only the phosphokinase reaction is observed. Later, however, the ATP concentration drops and the ^{32}P F1 activity rises so that the phosphatase reaction becomes progressively more important and eventually dominates. The kinetics can be predicted qualitatively by a simple mathematical model based on these assumptions (H. R. M., unpublished). As would be expected, increasing the initial ^{32}P -ATP: nucleus ratio shifts the position of the maximum incorporation to longer times (Fig. 2).

P. polycephalum nuclei were isolated at defined stages of the naturally synchronous mitotic cycle and their net phosphorylating activity measured. Figure 2 shows the results obtained with nuclei isolated in S phase or in G2 phase. There is clearly a large increase in net phosphorylating activity in G2 phase. Figure 3b summarises the results obtained with nuclei isolated from many points in the mitotic cycle. The F1 phosphorylating activity increases steadily through the mitotic cycle from a minimum near metaphase to a maximum in late G2 phase, 1.75 h before metaphase, and then falls very rapidly to the minimum near metaphase. This pattern is quite different from that observed for most *P. polycephalum* enzymes⁹. The increase in

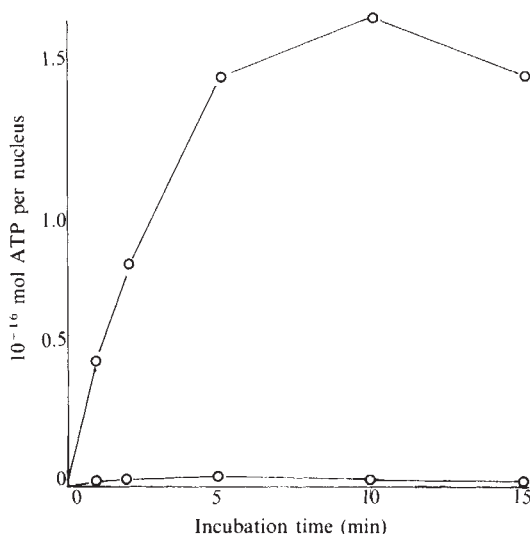


Fig. 2 Histone phosphorylating activity in G2 and S phases. Synchronous plasmodia of *P. polycephalum* were cultured on filter paper⁴⁵ and the stages of the mitotic cycle (M) were determined by microscopic examination of glycerol: ethanol fixed smears under phase contrast microscopy⁴². The assay was performed as described in the caption to Fig. 1 except that the number of nuclei was reduced to 1.7×10^7 per 1.5 ml and the first 25% TCA precipitate was washed and counted as described by Lake and Salzman¹⁰. Using the known specific activity of the ^{32}P -ATP and the known concentration of nuclei (counted in a haemocytometer) the results were expressed as mol phosphate transferred to F1 per nucleus. The F1 was present in excess (R. J. I., PhD thesis, in preparation). ●, Obtained using nuclei isolated 1 h after metaphase, that is in mid S phase; ○, obtained from nuclei isolated 1 h before metaphase, that is in late G2 phase.

phosphorylating activity precedes the increase in Pp F1 phosphate content as would be expected if the change in phosphate content were caused by the change in phosphorylating activity. Figure 3c shows the startling result of plotting the logarithm of the net F1 phosphorylating activity as a function of time in the cycle. It is clear that the increase in activity per nucleus follows a pure exponential law from early S phase to late G2 phase. The extremely rapid decrease in activity just before metaphase also follows an exponential law but the time period is so short that other interpretations may be possible.

Table 1 Comparison with Mitotic Stimulation Data

Stage of donor plasmodium h before mitosis	Fraction of generation time	Acceleration of mitosis ³⁷ (h)	Phosphorylating activity mol ATP per nucleus $\times 10^{-21}$
7.00	0.82	0.42	30
4.50	0.56	0.42	100
3.00	0.35	1.08	210
1.25	0.15	0.92	110
0.33	0.04	0.67	50

F1 phosphate content

The temporal correlation between Pp F1 phosphate content and chromosome condensation before mitosis indicates that phosphorylation induces the necessary preliminary structural changes in chromatin³. This is supported by the observations, in mammalian systems, of high F1 phosphate content in metaphase chromosomes¹⁰ and in late G2 and mitotic cells from synchronised cultures¹¹, and by the very widespread correlation between F1 phosphate content and growth rate¹². The data on Pp F1 (Fig. 3a) show no increase in overall phosphate content during S phase. If, however, the data are recalculated to simulate the type of pulse labelling experiment that has been carried out in mammalian systems¹³, then a second peak of phosphorylation appears in S phase as well as the first peak in late G2 phase (H. R. M., unpublished). The second peak is due to the fact that histones are synthesised during S phase and some phosphorylation of new histone is required to maintain the low 'background' F1 phosphate content. There is no evidence that this phosphorylation is connected with DNA synthesis, although a connection is not ruled out.

Several *in vitro* studies have cast histone F1 in the role of a cross linking agent in chromatin¹⁴⁻¹⁸. Moreover, a reversible *in vitro* condensation of chromatin can be induced by salt changes and this process does not occur with F1 depleted chromatin¹⁹. Nuclear magnetic resonance studies of the salt-induced condensation have suggested that the lysines of the basic ends of F1 always remain bound to the remaining DNA: protein complex but that the central 'interacting segment' has at least two conformations, one producing dispersed chromatin and the other(s) producing condensed chromatin²⁰. It is interesting that the phosphate sites in *in vitro* phosphorylated F1 histone are situated in the basic ends of F1 and not in the 'interacting segment'^{20,21}. A model of chromatin condensation based on phosphorylation is presented in Fig. 4. It is consistent with the observed weaker binding of F1 to DNA in condensed chromosomes²². As Pp F1 phosphate content falls during mitosis it is proposed that F1 phosphorylation is the initial mechanism of condensation and that other mechanisms are involved later, possibly disulphide bridges between F3 histones²³. This proposal is consistent with the fact that metaphase chromosomes are stable while phosphokinase activity decays during metaphase arrest^{10,24}.

It has been proposed recently that F1 phosphorylation controls interphase chromosomal coiling⁴. It is difficult, however, to reconcile such a proposal with the lack of effect of F1 on this structure *in vitro*. Further, the model is based on an early result that euchromatin F1 is highly phosphorylated²⁵, while the

more recent substantial criticisms of the methods by which this result was obtained²⁶⁻²⁸ have not been discussed⁴.

Initiation of mitosis

P. polycephalum is an excellent model system for the study of the control processes involved in the initiation of mitosis and therefore mitotic cell division. A considerable weight of data has been accumulated describing the properties of a supposed 'mitotic trigger' substance or substances²⁹ but no serious suggestions have yet been made concerning the molecular nature of the 'trigger' except that it may be protein³⁰. Its predicted properties, however, are very close to the observed properties of the F1 phosphorylating activity in *P. polycephalum* nuclei as reported here. We propose, as a working hypothesis to be tested, that F1 phosphorylation is the initiation step for mitosis and that this step is 'triggered' or controlled by net F1 phosphorylating enzyme activity.

The 'mitotic trigger' has been observed directly by adding a homogenate of a plasmodium relatively near to mitosis to a growing plasmodium relatively far from mitosis. Significant stimulation was observed and the nearer the homogenised plasmodium was to mitosis the greater its stimulatory effect until a maximum was observed³⁰. These experiments have been criticised on the grounds that they were found to be difficult to reproduce and the possibility of the effect being due to a general growth factor has not been ruled out (J. Mohberg, unpublished).

The results are in general agreement with the more widely accepted fusion experiments and we are inclined to accept them in the absence of any well documented alternative observation. Our proposal predicts a stimulatory effect from plasmodia at all stages of the mitotic cycle with a maximum 1.75 h (0.2 of a generation time) before mitosis, in agreement with Oppenheim and Katzir³⁰. Table 1 shows the agreement between the observed acceleration of mitosis³⁰ and the net F1 phosphorylating activity of nuclei (interpolated from the data of Fig. 3). The results are consistent, within the probable experimental error, with the conclusion that the stimulating effect is proportional to the F1 phosphorylating activity, in strong support of our proposal.

A slightly less direct but more popular approach to this problem has been to use the fusion between plasmodia at different stages of the mitotic cycle. The reaction involves fusion of the cytoplasm³¹ and diffusion of nuclei from one cytoplasm to another³². In all fused plasmodia the nuclei all divide synchronously at a time intermediate between the division times in the homogenous controls³³⁻³⁶ unless one of the donor plasmodia is close to division, in which case nuclei from this plasmodium are not affected by the fusion^{32,37}. This 'point of no return' occurred less than 0.14 of a generation time before metaphase. Our model does not lead to an accurate prediction of the earliest 'point of no return' but it would be expected to occur not later than the time when the phosphate content of Pp F1 had risen well above the early G2 phase level. At 0.14 of a generation time before metaphase the phosphate content was three to four times the early G2 phase level, two thirds of the total change having already occurred. This is clearly consistent with the observed earliest 'point of on return' which is thus probably associated with the phosphate content of Pp F1.

The stimulation or delay of mitosis observed with plasmodia fused at other times is probably associated with the cytoplasmic synthesis of F1 phosphokinase or its activator or repressor. The fusion experiments strongly implicate the cytoplasm³³ and ultraviolet irradiation experiments have been interpreted in the same way³⁷. Our model thus predicts that the stimulation of mitosis should increase with the time difference between the fused plasmodia, in agreement with Chin *et al.*³². In fact, if the stage of the fused plasmodium corresponds to the stage that would have the same mean level of F1 phosphorylating activity as the fused plasmodium has immediately after fusion, then the stimulation can be predicted directly (Fig. 3c), with no arbitrary

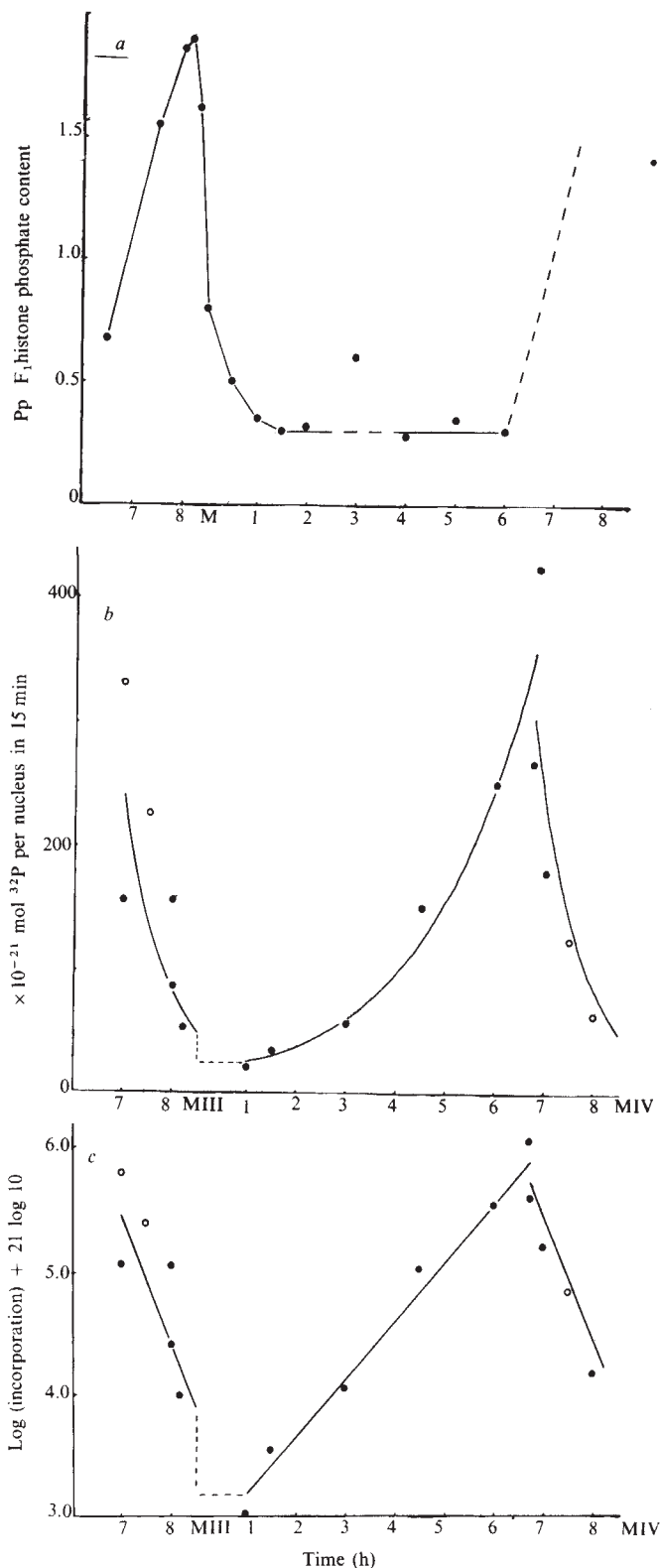


Fig. 3 Variations during the mitotic cycle. *a*, Phosphate content of Pp F1 on an arbitrary scale³; *b*, net F1 phosphorylating activity of *P. polycephalum* nuclei determined as described in the caption to Fig. 2. ●, Results obtained with 1.7×10^7 nuclei per 1.5 ml; ○, obtained with different concentrations of nuclei. The incorporation was assumed to be proportional to the concentration of nuclei. —, The equation; incorporation = $15 \times 10^{-21} \exp(0.47t)$, $1 < t < 6.75$ (Equation 1); incorporation = $340 \times 10^{-21} \exp(-1.04t)$, $6.75 < t < 8.3$; where t is time in h after the previous metaphase. - - -, Extrapolation obtained assuming the incorporation per nucleus falls by 50% at division. *c*, The data of *b* plotted on a logarithm scale. The straight lines, equations above, were fitted by least squares. The regression coefficients are $r = 0.99$, $1 < t < 6.75$; and $r = 0.79$, $6.75 < t < 8.5$.

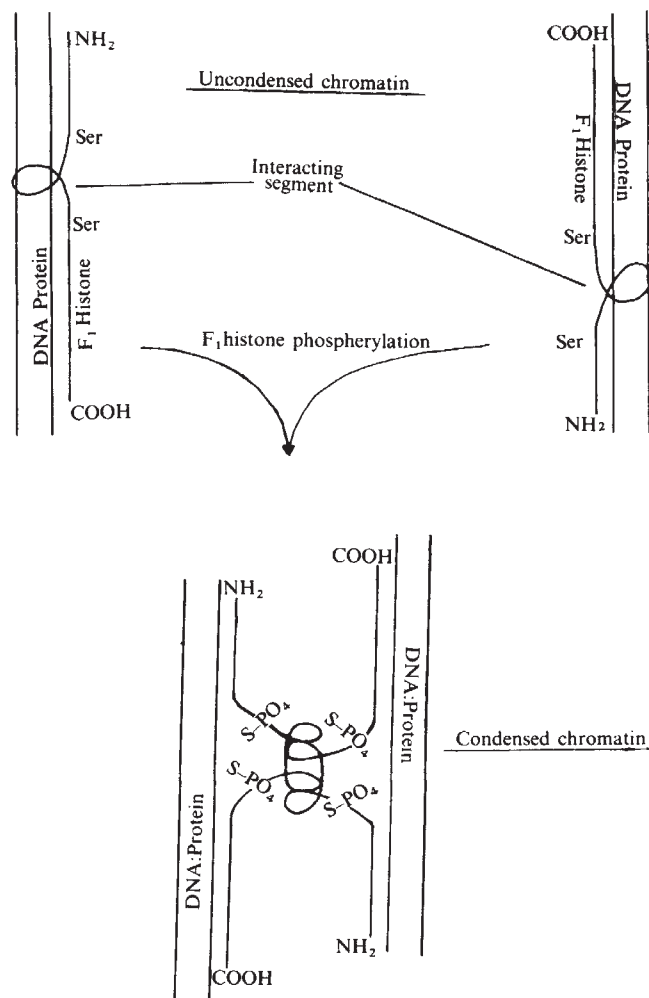


Fig. 4 Condensation of chromatin before metaphase. S-PO₄ and ser are the phosphorylation sites on F1; ser represents the nonphosphorylated form and S-PO₄ represents the phosphorylated form. The diagram is not intended to specify the precise details of the interactions, although a convincing series of inter- and/or intra-molecular attractions and repulsions are present²⁰. In interphase (uncondensed) chromatin it is proposed that the 'interacting segment' of F1 is loosely bound to DNA:protein. Phosphorylation of the serines at the ends of this segment would tend, due to repulsion from DNA phosphates, to lift the interacting segment away from DNA:protein. Such a tendency would increase the probability of an intermolecular interaction between F1 histones as shown above and such interactions would bring about the observed chromosome condensation. The data at present available do not rule out similar models involving only intramolecular conformational changes, nor is it essential that the phosphorylation sites be exactly where shown.

constants being required. The calculation of predicted mitosis stimulation for the times investigated by Chin *et al.*³² shows (Table 2) that the calculated value is always the same as the observed value within the experimental error; clear support of our proposal. The strength of the support is limited by the accuracy of the data. The effect of elevated temperature shocks on the timing of mitosis in *P. polycephalum*³³ is also in agreement with our proposal. *In vitro* pre-incubation for 30 min at 37° C reduces the F1 phosphorylating activity to about 50% of normal (R. J. I., unpublished). Thus our proposal predicts that the effects of temperature shock should be minimal in early S phase, rising exponentially to a maximum about 1.75 h before metaphase and then falling sharply. This is exactly the pattern observed by Brewer and Rusch³⁴. They also observe a transition point at 16 min before metaphase where temperature shock prevents mitosis altogether instead of just delaying it. This

Table 2 Comparison with Plasmodial Fusion Data

Unfused control time to mitosis*	Unfused time difference between plasmodia	Observed stimulation of mitosis ³⁵	Calculated stimulation of mitosis
		±0.04	
0.49	0.34	0.21	0.22
0.51	0.30	0.22	0.19
0.67	0.40	0.26	0.27
0.58	0.31	0.20	0.20
0.42	0.13	0.09	0.07
0.38	0.06	0.05	0.03
0.47	0.17	0.10	0.10
0.56	0.18	0.10	0.11
0.69	0.29	0.16	0.18
0.52	0.10	0.09	0.06
0.62	0.18	0.13	0.11
0.69	0.25	0.13	0.16

*All times are fractions of one generation time.

corresponds in time to the onset of the proposed second stage of chromosome condensation.

The proposal does not make any particular predictions about the levels of protein and nucleic acid synthesis required for the initiation of mitosis. It is worth noting, however, that the inhibition of protein synthesis does not prevent the completion of mitosis if the inhibitor is added in late prophase^{36,40} just after the peak of Pp F1 phosphate content⁸, thus supporting our proposal. The initiation of mitosis seems to be dependent on previous DNA synthesis³⁷ but not actinomycin D-sensitive RNA synthesis⁴¹.

We conclude that phosphorylation of F1 initiates chromosome condensation which is then completed by a second process; that this phosphorylation is controlled by net F1 phosphorylating enzyme activity; and that these processes represent the two main steps in the initiation and control of initiation of mitotic cell division. These are consistent with the experimental data available, but to test these proposals further an attempt is being made to stimulate mitosis by adding purified phosphokinase to growing plasmodia of *P. polycephalum*. Other tests would be to study F1 phosphokinase activity in fused, temperature shocked, drugged or irradiated plasmodia. If the proposals are correct it may be possible to inhibit mitotic cell division without affecting other normal cell functions using F1 histone phosphatase. This would apply to cancer as well as normal cells¹².

Finally, it should be pointed out that although the evidence for these proposals is widespread, it is all, so far, circumstantial and more direct experiments are needed to confirm or deny them.

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Evolution in Action

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The evolution of enzymes in bacteria is studied by introducing novel substrates and selecting strains that have mutated to be able to use these substrates.

THE present day bacteria possess enzymes which are the end products of millions of years of evolution, but there is no reason to suppose that their evolutionary potential is at an end. Bacteria that live in soil and water encounter a vast array of organic compounds, arising from natural bio-synthetic processes, which can be utilised as growth substrates. These have been augmented in recent years by the products of the chemical industry, thereby creating selection pressures for the evolution of enzymes for the degradation of man-made molecules. Catabolic pathways are known for such compounds as camphor, naphthalene, phenol, catechol, cresols, alkanes and also for synthetic detergents such as benzene sulphonate, and herbicides, for example, 2,4-

dichlorophenoxyacetate (2,4-D). For many of these catabolic pathways the detailed enzyme reactions have been established and in some cases the genetic determinants have been analysed¹. The ability to metabolise organic compounds depends on a number of factors including the possession of enzymes with the appropriate specificities, transport systems to get the potential growth substrate into the cell and regulatory mechanisms that can be activated when required.

We have taken a single enzyme, an inducible aliphatic amidase of *Pseudomonas aeruginosa*, and shown that it is possible to evolve amidases with altered substrate specificities which enable mutants to utilise amides that do not support growth of the wild-type parent²⁻⁴ (Table 1). These changes in enzyme specificity involve very few changes in the primary structure of the enzyme protein and arise from a small number of single-site mutations. A comparison of a number of independent isolates of *P. aeruginosa* showed that they were able to utilise the same amides for growth; the substrate specificities of their amidases, and the apparent K_m values obtained for the various amides, were the same within the limits of the measurements made. Thus, the substrate specificity of the aliphatic amidases of wild-type strains of *P. aeruginosa* seems to be a stable character for this species, although it can be changed in the laboratory under the appropriate selection pressure.

The first systematic studies of the evolution of strains with the ability to utilise novel growth substrates, were made by

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Wood and Mortlock and their colleagues who investigated the growth of various bacterial species on pentoses and pentitols⁶. They found that the most common mutation which allowed the use of a novel growth substrate was a mutation conferring constitutivity on an enzyme that already had some affinity for the compound. The system was thus freed from the constraints of inducer specificity; since constitutive mutants can produce large amounts of enzymes (2% or more of the total cell protein), the amount of the enzyme compensated for its feeble activity. In one case a regulatory mutation produced a mutant with altered inducer specificity that could be induced by the novel growth substrate⁷. This is a more satisfactory biological solution since derepressed strains are at a growth disadvantage compared with wild-type strains under growth conditions which do not require the derepressed enzyme⁸. This is because they expend energy on synthesising the constitutive enzymes even when they are of no metabolic value to the cell. Mutations in the structural genes to produce enzymes with increased affinities for their novel substrates, have been described for L-fucose isomerase⁹ and ribitol dehydrogenase¹⁰. The evolutionary sequence established by Wu, Lin and Tanaka¹⁰ for an increased rate of growth of *Klebsiella aerogenes* with xylitol was first, a mutation resulting in a constitutive ribitol dehydrogenase, second, a mutation increasing the affinity of ribitol dehydrogenase for xylitol, and third, a mutation which allowed

weight of about 200,000 and is made up of six identical subunits.¹¹

The successful isolation of mutants producing amidases with altered substrate specificities depended on exploiting the regulatory characteristics of the enzyme, and using a variety of different amides in the selection media. Amides can be used as nitrogen sources, carbon sources or both at the same

Table 2 Amides and inducers, enzyme substrates and growth substrates for wild type *Pseudomonas aeruginosa* PAC1

Amides	Relative rates of hydrolysis	Relative rates of induction	Amide analogue repression	Availability as growth substrate
Formamide	10	7	+	—*
Acetamide	100	100	.	+
Propionamide	300	50	.	+
Butyramide	2	0	+	—
Valeramide	0	0	+	—
Phenylacetamide	0	0	+	—
Acetanilide	0	0	+	—

* Formamide can be used as a nitrogen source for growth but not as carbon source since C₁ compounds are not utilised by this strain.

Table 1 *Pseudomonas aeruginosa* amidase mutants shown in Figs 1 and 2

Strain No.	Series No.	Phenotype	Genotype
PAC1	Wild	Ind A ⁺ B ⁻	<i>amiR⁺amiE⁺</i>
PAC111	C11	Con A ⁺ B ⁻	<i>amiR11amiE⁺</i>
PAC153	F6	F-ind A ⁺ B ⁻	<i>amiR43amiE⁺</i>
PAC142	L10	Con A ⁺ B ⁺ But-r Crp-r	<i>amiR33amiE⁺crp-7</i>
PAC128	CB4	Con A ⁺ B ⁺ But-r	<i>amiR11,37amiE⁺</i>
PAC351	B6	Con A ⁺ B ⁺	<i>amiR11amiE16</i>
PAC366	AI3	Con A ⁺ B ⁻ AI ⁺ Crp-r	<i>amiR33amiE56crp-7</i>
PAC326	LAm1	(Ind)A ⁻ (Crp-r)	<i>amiR33amiE34crp-7</i>
PAC392	PhF1	ConA ⁽⁺⁾ B ⁺ V ⁺ Ph ⁺	<i>amiR11,37amiE82</i>
PAC377	PhB3	Con A ⁻ B ⁺ V ⁺ Ph ⁺	<i>amiR11amiE16,67</i>
PAC353	V2	Con A ⁻ B ⁺ V ⁺	<i>amiR11amiE16,23</i>
PAC356	V5	Con A ⁻ B ⁺ V ⁺	<i>amiR11amiE16,26</i>
PAC360	V9	Con A ⁻ B ⁺ V ⁺	<i>amiR11amiE16,30</i>
PAC391	PhA1	Con A ⁽⁺⁾ B ⁺ V ⁺ Ph ⁺	<i>amiR33amiE34,81crp-7</i>
PAC388	PhV1	Con A ⁻ B ⁺ V ⁺ Ph ⁺	<i>amiR11amiE16,30,78</i>
PAC389	PhV2	Con A ⁽⁺⁾ B ⁺ V ⁺ Ph ⁺	<i>amiR11amiE16,30,79</i>
PAC398	IB10	Ind A ⁺ B ⁻	<i>amiR⁺amiE16</i>
PAC308	Am8	(Ind) A ⁻ B ⁻	<i>amiR⁺amiE8</i>

The genotype is given as *amiR*, amidase regulator gene; *amiE*, amidase structural gene; *crp*, gene(s) concerned with catabolite repression. The phenotype is given for growth; +, good growth; (+), trace growth; —, no growth on minimal agar plates containing as carbon sources A, acetamide; B, butyramide; V, valeramide; AI, acetanilide and as nitrogen source only, Ph, phenylacetamide (refs 2–4, 13, 14 and J. E. Brown, J. L. B., P. H. C., and M. D., in preparation).

xylitol to be transported more rapidly into the cell. In a detailed investigation (unpublished) of selection in continuous culture, B. S. Hartley, P. W. J. Rigby, and B. D. Burleigh, jun., found that mutations leading to gene amplification also increased the rate of growth of this organism with xylitol.

The aliphatic amidase of *P. aeruginosa* has a well defined specificity for amide hydrolysis and also a well defined inducer specificity (Table 2). The two amides that can be utilised for growth by the wild-type strain are acetamide and propionamide and the corresponding acids can be readily metabolised. *P. aeruginosa* grows well in simple salt media and can be grown on a scale which provides sufficient of the enzymes for protein studies. The amidase has a molecular

time. The altered amidase mutants shown in Table 1 were all selected from plates in which the following amides provided part or all of the growth requirements: butyramide, valeramide, phenylacetamide and N-phenylacetamide. No mutants producing altered enzymes could be isolated directly from the wild-type strain and the regulatory phenotype of the parental strains was critical in determining whether or not a particular class of altered enzyme mutant could be obtained by this simple positive selection method. The strategy adopted in this attempt at experimental evolution was based on a detailed study of the mechanism of regulation of amidase synthesis^{12,13}. As we discuss in detail later, some altered enzyme mutants were isolated only from parental strains which were maximally derepressed for amidase synthesis, while others could be isolated only from a particular class of repressible mutants.

It can be seen from Table 2 that butyramide cannot be used as a growth substrate by the wild-type strain although it is hydrolysed by amidase at about 2% of the acetamide rate. Butyramide is unable to induce amidase synthesis in *P. aeruginosa* and furthermore, acts as a competitive inhibitor of induction by both substrate and non-substrate amides. It is thought to act by combining with the regulator binding protein produced by the amidase regulator gene at the inducer-binding site, without producing the conformational change necessary to enable expression of the amidase structural gene. This imposes an additional constraint on the regulation of amidase synthesis. Other amides act in this way and we refer to them as amide analogue repressors¹².

Butyramide-utilising mutants were isolated spontaneously from the wild type on butyramide plates at a frequency of about 10⁻⁶ and all were constitutive, producing wild-type A amidase (CB group). This is another example of a mutation to constitutivity conferring the ability to utilise a poor substrate. But, not all constitutive mutants grow on butyramide. Earlier, we had isolated constitutive mutants from succinate+formamide (S/F) plates, with succinate providing the carbon source and formamide the nitrogen source (C group). Formamide is a poor inducer and a poor substrate for the amidase; the wild type grows very slowly on this medium and constitutive mutants are readily isolated. About half of the constitutive mutants obtained from S/F plates are butyramide-positive in phenotype, and about half are butyramide-negative. The constitutive mutants that were unable to grow on butyramide were found to have retained the sensitivity to amide analogue repression which had been exhibited by the wild-type strain¹⁴.

The first of the mutants producing an altered enzyme was obtained by selection on butyramide plates after mutagenesis of strain C11, a constitutive mutant with a butyramide-negative phenotype². This B group of mutants retained the regulatory phenotype of the parent strain, and amidase synthesis was repressed by amide analogues. All the B group mutants seemed to produce the same amidase and mutant B6 was used to isolate the new enzyme, B amidase (Tables 1 and 3). B amidase differs from the wild-type A amidase in electrophoretic mobility, but is thermostable and gives complete cross reaction with antiserum to A amidase. B amidase is only slightly less active towards acetamide than is A amidase, and has increased activities for both propionamide and butyramide. The K_m for butyramide is decreased almost ten-fold and the V_{max} is increased ten-fold; the relative specific activities of B amidase for acetamide and butyramide are 100:30 whereas for A amidase they are 100:2. Although butyramide is still capable of repressing amidase synthesis in the B group of mutants, the rate of hydrolysis of butyramide by the altered enzyme removes it very rapidly from the growth medium¹⁴.

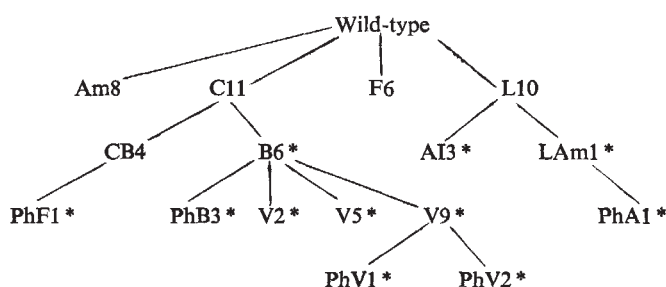


Fig. 1 Family tree of *Pseudomonas aeruginosa* amidase mutants derived from strain PAC1. Those producing mutant enzymes are marked with an asterisk, others are regulatory mutants or devoid of activity with all amides tested. Details of phenotypes and genotypes in Table 1.

A further mutational step in this evolutionary pathway produced the V class of mutants which are able to utilise valeramide for growth (Table 1 and Fig. 1). The V amidases are a heterogeneous group and differ among themselves in substrate specificities and in thermal stability. The specific activities of all these enzymes were much lower than those of the wild-type A amidase or the mutant B amidase². Within the bacterial cells they were stable, however, and this group of mutants grew quite well with valeramide both in liquid medium and on plates. One of the enzymes, V9 amidase, was cold labile but was successfully purified at room temperature. This class of mutant is expected to yield useful information on the amino acid substitutions affecting enzyme stability and the maintenance of catalytic activity.

The final class of mutants isolated by this route were two groups of phenylacetamide-utilising mutants, the PhB group and PhV1 and PhV2 (Table 1 and Fig. 1)⁴. All were derived from C11 through B6; the PhB group were derived in a single step from B6 while PhV1 and PhV2 were derived through V9. The amidases produced by these mutants are much less active than the wild-type enzyme, whether measured in whole cells or in extracts, but good growth occurs with phenylacetamide as the nitrogen source for growth. *P. aeruginosa* does not utilise the phenylacetate produced by hydrolysis of phenylacetamide so that an additional carbon source must be supplied.

A second route was also effective in obtaining mutants capable of utilising phenylacetamide. Strain CB4, one of the constitutive mutants with a butyramide-positive phenotype,

was chosen as a potential parent strain; it was thought that its resistance to amide analogue repression might allow growth on phenylacetamide of mutants producing altered enzymes even if their specific activities towards phenylacetamide were not very high. Strain CB4 gave rise in a single mutational step to PhF1 (Fig. 1); the PhF1 amidase is one of the more unstable of the mutant amidases isolated so far.

Before mentioning the final evolutionary pathway which led to a phenylacetamidase, it is necessary to consider a further control which is imposed on amidase synthesis. Like many catabolic enzymes, amidase is subject to catabolite repression and one of the most potent of the repressing metabolites is succinate. In the presence of succinate as the carbon source and a low concentration of lactamide (a good inducer but a poor substrate) as nitrogen source, the wild type grows very slowly. This makes it possible to isolate mutants which are resistant to catabolite repression. Those that are both constitutive and resistant to catabolite repression, synthesise amidase to form about 10% of the total cell protein when grown in appropriate media. One of these mutants, L10, is used for routine preparation of A amidase, but is also useful as a parent strain for isolating mutant amidases. The large amounts of amidase protein produced by this strain means that the threshold of activity which allows growth on a substrate amide can be achieved, even with an enzyme of very low specific activity. A class of mutants producing defective amidases were isolated from strain L10 as parent. One of these, strain Lam1 (see Table 1 and Fig. 1), was the parent of the phenylacetamide-utilising mutant PhA1. The route by which PhA1 was derived, suggests that it has two separate mutational sites. Since PhA1 grows very slowly on acetamide it could, however, be regarded as a partial revertant and the mutation allowing growth on phenylacetamide might have occurred at the same site as the original mutation. The PhA1 amidase is very thermolabile and in this respect it resembles PhF1.

The most interesting of the mutant enzymes isolated so far is one that hydrolyses acetanilide (N-phenylacetamide) and allows growth with acetanilide as carbon source (Table 1). Mutant AI3 can also utilise acetanilide as nitrogen source, but growth is then slower since the hydrolysis product, aniline, is metabolised rather poorly. This mutant was derived directly from the catabolite repression-resistant mutant L10, which is also constitutive and resistant to amide analogue repression. The change in substrate specificity to allow the enzyme protein to accommodate the benzene ring, results in a decrease in the acetamidase activity, but this mutant is still capable of good growth on acetamide plates³. But while the parent strain L10 grows very well on butyramide plates, the mutant AI3 gives only trace growth since the specific butyramidase activity of the altered enzyme is very

Table 3 Apparent K_m values ($\times 10^{-3}$ M) for wild type and mutant amidases

Substrate	Enzymes					
	A	B	AI	PhB3	PhV1	PhF1
Acetamide	19	15	53	NP	NP	120
Propionamide	55	10	208	NP	NP	ND
Butyramide	500	74	ND	180	200	38
Phenylacetamide	NP	NP	NP	2	3	1

The enzyme was assayed by the acyl-group transfer reaction with hydroxylamine as acceptor (500 mM)¹². ND, not determined; NP, not possible as no activity could be detected for that amide.

greatly reduced. The AI3 amidase can also attack *p*-substituted acetanilides, including *p*-nitroacetanilide, *p*-bromoacetanilide and *p*-chloroacetanilide (M. J. S. and P. R. B., in preparation). The AI3 enzyme gives a complete cross reaction with antiserum to A amidase and is stable in

the cell-free state. It differs from both A and B amidases in electrophoretic mobility and the purified enzyme loses 40% of its activity on heating for 10 min at 60° C, while the A and B amidases are unaffected by this treatment. The difference between the AI3 amidase and the A amidase resides in the substitution of a single amino acid residue; a threonine residue of the wild-type enzyme is replaced by an isoleucine residue in the mutant AI3 enzyme³.

Table 3 gives some of the apparent K_m values we have obtained for the wild-type and mutant amidases. The only case in which it was possible to compare K_m values for a novel growth substrate with both the parental and mutant enzyme was for butyramide with A and B amidases; the apparent K_m value for butyramide of A amidase was extremely high, 500 mM, while for B amidase it was 74 mM. Butyramide is one of the best substrates for the Ph group of enzymes and it can be seen that different K_m values for butyramide were obtained with the PhB3, PhV1 and PhF1 enzymes, which also differ from one another in other respects.

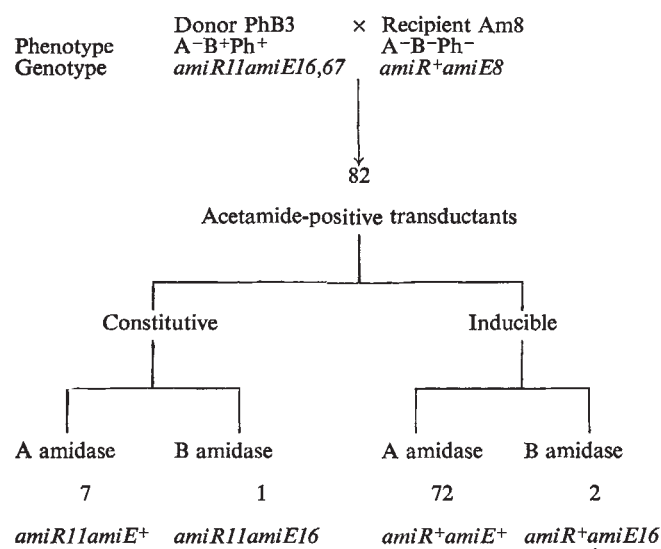


Fig. 2 Transductional cross between two amidase mutants which gave rise to some recombinants that produced the mutant B amidase and were inducible*. Other recombinant classes not shown above were presumed to be acetamide negative in phenotype.

No measurements with phenylacetamide could be made with amidases other than the Ph group since they had no detectable activity with this amide. It was found that the purified B amidase had trace activity for acetanilide and it is probable that this is also the case for the A amidase but no measurements have been made. The values given in Table 3 were all obtained using the acyl-group transferase reaction for assaying amidase activity¹².

The mutant amidases all have the same molecular weights and the same number of subunits; they all cross react with antiserum to the wild-type enzyme although some of the less stable mutant enzymes give rather diffuse bands. These less stable enzymes, particularly PhF1, PhV1 and some of the V amidases, should be of considerable value in looking at the subunit interactions of this protein. The existence of a series of very closely related enzymes also offers opportunities for exploring enzyme mechanisms by varying the enzyme structure rather than the substrate structure, and A, B and AI3 amidases are very suitable for this purpose.

Evolution of novel activities for *P. aeruginosa* amidase has

been achieved experimentally by several different paths and it is not possible to say whether any of these routes might be followed in nature. There would be, for example, very little advantage for *P. aeruginosa* to have acquired a gene duplication and then to have evolved a phenylacetamidase. *P. aeruginosa* is unable to utilise phenylacetate, and shortage of carbon compounds, not nitrogen compounds, limits growth in the natural environment. We have, however, found that strains of *P. putida*, which are able to utilise phenylacetate, have evolved a phenylacetamidase which allows growth on plates with phenylacetamide providing both carbon and nitrogen source¹⁵. The *P. putida* phenylacetamidase is inducible by phenylacetamide as would be predicted. All our altered enzyme mutants are constitutive and are not yet suitable candidates for a natural ecosystem. They might be considered as strains in transition, for which the next step would require a mutation to allow them to be induced by the new substrate. We have constructed one inducible strain producing an altered enzyme, by recombination between PhB3 and an amidase-negative mutant (Fig. 3) (J. E. B., J. L. B., P. H. C., and M. Day, in preparation). The recombinant has a wild-type regulator phenotype and produces B amidase, but since it cannot be induced by butyramide this strain grows no better on butyramide than the wild-type. It does, however, open the possibility for new inducible strains to arise by recombination of various mutants.

Evolution by gene duplication is widely held to have played a major part in the evolution of enzymes¹⁶. Koch¹⁷ suggests that to allow time and opportunity to acquire a sufficient number of mutations the duplicate gene should be retained unexpressed for a long period. We have shown for one enzyme at least, that very few mutations are needed to make considerable changes in substrate specificity which enable the organism to utilise new growth substrates. In *Pseudomonas* there is an additional genetic characteristic which may hasten the spread of an advantageous mutation through a population. Genes for several catabolic pathways have been found to be carried on transmissible plasmids; camphor¹⁸, octane¹⁹, salicylate²⁰ and naphthalene²¹ plasmids have been identified. Genes for catabolic enzymes may be exchanged between chromosomes and plasmids, and new mutations offering a selective growth advantage may be spread much more rapidly through the microbial population than the classical interpretation of mutation and selection would predict.

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Sequence divergence of mammalian globin messenger RNA

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The rate of nucleotide change between globin genes for mouse, rabbit, rat and man is considerably slower than that of non repeated DNA, suggesting that selection pressure is less stringent on the bulk of the genome than on genes coding for polypeptides.

THE role of the very large amount of DNA in the haploid mammalian genome is still obscure. There are simple sequences present at very high multiplicity which are not transcribed, of which the best studied is the satellite DNA of mouse. The bulk of the mammalian genome consists of a mixture of sequences many of which are present only in single copies, while others occur several hundred or thousand times. It is known that among the single-copy sequences are many of the genes coding for specific proteins, although some reduplicated sequences are also transcribed.

The function of the greater part of the mammalian genome is not clear. It has been suggested, from a consideration of the rate of accumulation of neutral mutations between species, that a large proportion of the genome may not code for protein^{1,2}. A similar conclusion has been reached from a consideration of possible models for the control of gene expression³⁻⁶. A comparison of the rate of divergence of a single gene with the rate of divergence of the single-copy genome would indicate any difference in mutation rates between nucleotide sequences expressed as protein sequences and the total non-repeated DNA.

Sequence of globin gene

The nucleotide sequence of the globin gene is particularly suitable for a study of divergence, since it is known that the mRNA is complementary to, and thus synthesised from, sequences reannealing with the nonrepetitive fraction of the total DNA. These sequences are present as single, or at most two or three, copies⁷⁻¹⁰. Thus any changes in the nucleotide sequence of the gene will be reflected necessarily in the sequence of the globin mRNA. The single-copy DNA can be isolated using hydroxylapatite after allowing the rest of the DNA to reanneal, and its divergence between different mammalian species can be investigated and has been previously reported^{11,12}.

Globin mRNA can be isolated in pure form from reticulocytes of animal species¹³⁻¹⁵. As the amino acid sequences of globins from many different mammals are known, both the maximum and minimum divergence of the corresponding nucleotide sequences can be predicted, taking into account

both amino acid differences between species and also possible third position changes in synonymous codons.

The rate of nucleotide change in the globin mRNA, and thus in the globin gene, has been determined by studying the extent of specific base sequence pairing (cDNA:RNA hybridisation) between a complementary DNA copy (cDNA) of the mRNA made with tumour virus reverse transcriptase and the mRNA of the same or different species. Nucleotide changes within the hybridised sequence can also be studied by determining the depression of the melting temperature of the hybrid which such mismatching causes.

We report here the degree of sequence divergence between the globin genes for mouse, rabbit, rat and man. In this way the actual rate of nucleotide change with evolution of this single functional gene has been found to be considerably slower than that of the total non-repeated DNA, although greater than the minimum which would be consistent with the differences in the amino acid sequence.

Globin mRNA was prepared from rabbit, rat and mouse reticulocytes by affinity chromatography to poly U-Sepharose¹⁴⁻¹⁶. In each case the mRNA gave a single major component at 9S on polyacrylamide gel electrophoresis. The human mRNA was prepared using similar techniques from circulating blood of an adult patient with chronic non-thalassemic anaemia. It was not possible to determine the electrophoretic purity of the small amount of human mRNA available.

Reverse transcriptase

Reverse transcriptase was prepared from avian myoblastosis virus through the CM-Sephadex purification step¹⁷. cDNA was prepared using purified mouse globin mRNA as template and oligo(T) as primer and was fractionated on an alkaline sucrose gradient to give a product of average molecular weight 120,000 (approximately 320 nucleotides)^{7,18}. The cDNA was labelled with ³H-dTTP and ³H-dCTP (Radiochemical Centre, Amersham) and was at a final specific activity of 5×10^7 d.p.m./ μ g⁻¹.

The cDNA was hybridised in solution to a vast excess of mRNA. The hybridisation was analysed both by measuring resistance to S1 single strand specific nuclease¹⁹ and binding to hydroxylapatite²⁰. The stability of the hybrids was measured by the proportion eluted from hydroxylapatite with increasing temperature. Hybridisation of cDNA to mouse, rat and rabbit mRNA was for 18 h or less, to minimise the possibility of degradation of RNA affecting the rate; with human mRNA some hybridisations had to be continued for

longer periods of up to 42 h because of the small amounts of RNA available. All points were determined in duplicate or triplicate.

TABLE 1 Homology between mouse cDNA and mouse, rat, rabbit and human globin mRNAs, as measured on hydroxylapatite.

Source of RNA	$[mRNA] \times t$	% Reassociation	% Reassociation relative to mouse	T_m
Mouse	7.5×10^{-4}	80%	100%	76
Rat	2.3×10^{-3}	80%	100%	74
Rabbit	3.5×10^{-3}	50%	63%	69.5
Human	1.7×10^{-3}	30%	38%	69.5

Data are compiled from Figs 1 and 2.

The kinetics of annealing between mouse globin cDNA and mouse, rabbit, rat and human globin mRNAs as measured with hydroxylapatite are shown in Fig. 1. The half times and extent of reannealing are given in Table 1. Approximately 5% of the cDNA behaves as if double stranded even in the absence of driver RNA, and 20% fails to reanneal even for homologous hybridisation, as found previous for globin cDNA^{7,18}. In principle if both cDNA and mRNA are intact and even a segment of the molecules anneals, one should measure 100% reassociation between mouse cDNA and rabbit or human mRNA when the hybrids are analysed on

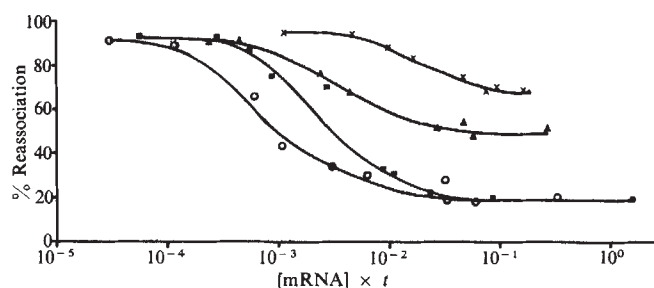


FIG. 1 Kinetics of reassociation of mouse globin cDNA with globin mRNA from mouse, rat, rabbit and human. Reassociations were in 0.12 M phosphate buffer, pH 6.8, 0.06% SDS, at 60°C. Hybrids were analysed on 0.1 ml hydroxylapatite columns at 60°C as described by Laird *et al.*²¹ except that reassociated molecules were eluted with 0.3 M phosphate buffer, 0.06% SDS, 2000 c.p.m. of cDNA were used for each point. This was reacted to RNA which was in 10^4 molar excess over the cDNA. $[mRNA] \times t$ (mol s^{-1}) is calculated from the initial concentration of RNA and the time of incubation²⁰. ○—○, Mouse; ■—■, rat; ▲—▲, rabbit; ×—×, human.

hydroxylapatite. The failure to do this indicates heterogeneity in the distribution of divergence in the population of molecules; either because they are smaller than the total message, or because a portion has accumulated more sequence divergence.

The melting profiles obtained for hybrids formed after annealing was complete are shown in Fig. 2. The melting temperatures obtained are given in Table 1. Each melting profile was determined at least twice.

S1 nuclease sensitivity of the hybrids was also determined after annealing was complete (Table 2), under comparable conditions of stringency to those used in the hydroxylapatite experiments, as well as under conditions of lower stringency.

Sequence homology

The molecular weight of globin mRNA is approximately 220,000, of which 150,000 is coding sequence for the protein moiety. The cDNA copy used was approximately 60% of the length of the mRNA. The amino acid sequences for globin for the mammals studied differ by as much as 19% (Table 3). Since such changes can in most cases be generated by single base alterations, the minimum divergence predicted for nucleotide coding sequences is 7% for human and mouse (Table 3). But if all possible synonymous codon changes and maximum codon differences for amino acid substitutions are allowed a divergence of from 35% to greater than 40% in the coding region is predicted, and stable hybrids would not be formed under conditions employed for these experiments^{24,25}. The sensitivity of the interspecific cDNA-mRNA hybrids to S1 nuclease demonstrates that more divergence has occurred in the mRNA sequence than the minimum predicted from the amino acid differences.

It is also possible to compare the divergence of globin mRNA sequences with that of the total non-repeated DNAs of the species studied. The melting temperature (T_m) of a DNA-RNA hybrid decreases with increasing base mis-pairing, and the difference between the T_m for a homologous and a heterologous hybrid (ΔT_m) is a measure of the divergence between the nucleic acids studied. Using comparable hybridisation conditions to ours McConaughy and McCarthy¹¹ found the homology between mouse and rat unique DNA to be 45% as compared with 100% for globin mRNA sequences, with a ΔT_m of 14°C as compared with 2°C. The homology

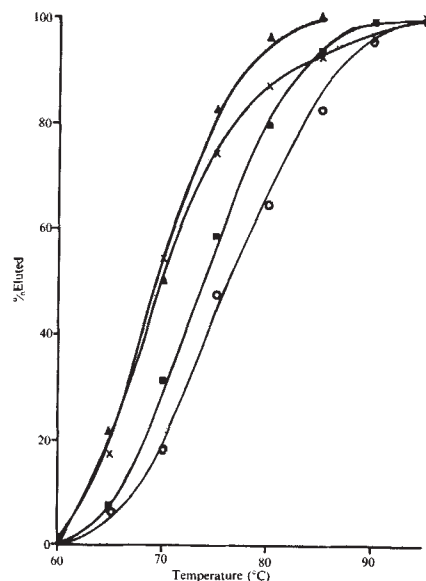


FIG. 2 Thermal dissociation of hybrids of mouse cDNA with globin mRNA from mouse, rat, rabbit and human. Hybrids were formed by reassociation to $[mRNA] \times t$ of greater than 10^{-1} as described in Fig. 1. The duplexes were bound to hydroxylapatite and dissociated as described by Laird *et al.*²¹. The mouse, rat, and rabbit curves were determined simultaneously under identical conditions using 10,000 c.p.m. of cDNA per curve. It was necessary to determine the human T_m separately, using 5,000 c.p.m. of cDNA. ○—○, Mouse; ■—■, rat; ▲—▲, rabbit; ×—×, human.

between mouse and guinea pig is only 6% with a ΔT_m of 16°C; it is likely that rabbit and human non-repeated DNAs are even less like mouse. Kohne¹² has shown that under less stringent conditions 13% of human non-repeated DNA will react with rat DNA and that 70% of non-repeated mouse

DNA will hybridise with rat DNA with a ΔT_m of greater than 15°C. The homology between globin mRNAs is much greater, demonstrating that different degrees of selection

TABLE 2 Homology between mouse cDNA and mouse, rat, rabbit, and human globin mRNAs as measured with S1 nuclease.

Source of RNA	Homology relative to mouse	
	Temperature of incubation 50° C	60° C
Mouse	100%	100%
Rat	97%	71%
Rabbit	79%	38%
Human	64%	32%
cDNA alone	15%	6%

Reassociations were performed in 0.18 M NaCl, 0.01 M Tris, pH 6.5 at the temperatures indicated to $[mRNA] \times t$ of greater than 10^{-1} in vast RNA excess. The reactions were cooled to 0°C and diluted to 0.18 M NaCl, 0.03 M sodium acetate buffer, pH 4.5, 10^{-5} M $ZnSO_4$, in the presence of 80 μg ml $^{-1}$ of *Escherichia coli* RNA. S1 nuclease was added and the digestions were carried to completion by incubation at the temperature of hybridisation¹⁹. Nuclease-resistant hybrids were precipitated with TCA²². 800–1,000 c.p.m. cDNA were used for each point. Results are normalised to mouse for which 80% of the input was nuclease resistant.

operate on this coding sequence than on the genome as a whole. If this slow rate of divergence relative to total non-repeated DNA sequences is characteristic for other mRNAs, it may be concluded that much of the non-repeated DNA is not in sequences coding for proteins.

cDNA used in these experiments was copied from purified mouse globin mRNA containing both α and β mRNA. The amino acid sequences of the mouse α and β globins are sufficiently different that the nucleotide sequences coding for them will not hybridise. The degree of amino acid divergence between species is similar for both chains (Table 3). It is

TABLE 3 Interspecific homology of mouse, rat, rabbit and human

Amino acid sequences of globin chains ⁽²³⁾	Amino acid % difference	Predicted globin mRNA coding sequence difference	
		Minimum	Maximum
Mouse α /rabbit α	19%	7%	40%
Mouse α /human α	13%	5%	35%
Mouse β /rabbit β	19%	7%	40%
Mouse β /human β	18%	7%	40%
Nonrepeated DNA			
	% cross-reaction	ΔT_m	
Mouse and rat ¹¹	45%	14° C	
Rat and human ¹²	13%	Not determined	
Mouse and human or rabbit (calculated from ref. 11)	<6%	>15° C	

assumed that the divergence seen between the nucleotide sequences similarly reflect changes in mRNAs for both chains. It is possible that a greater degree of nucleotide change has occurred in one chain than the other, as suggested by Housman *et al.*²⁶, but this is not apparent in the reassociation kinetics or melting profiles, which would be biphasic were divergence introduced into one of the two mRNAs preferentially. Leder *et al.*²⁴ have suggested from data similar to ours that the lack of homology between mouse and rabbit globin mRNAs may be accounted for by rapid divergence of non-coding sequences present in the globin mRNA. Since the cDNA is transcribed from the 3'-end of the mRNA it is possible that as much as 43% of the transcript could be non-coding

sequences, in the unlikely event that all of the extra nucleotides of globin mRNA are at the 3'-end of the molecule. But the close homology between mouse and rat globin mRNAs argues that there is little divergence in this case even for such cDNA sequences as may be derived from non-coding regions.

The data are equally consistent with a considerable amount of synonymous codon change having occurred in the message. For human globin cDNA, it has been reported that although the transcript is of small size, it contains a full representation of the sequences of the coding region of the mRNA²⁷.

Results similar to these have been obtained for sea urchin histone mRNAs^{2,25} and neutral fixation of synonymous codon changes has been proposed to explain the interspecific divergence observed²⁵.

While the function of non-repeated DNA is still obscure, it is clear that the globin gene is conserved more stringently than is total non-repeated DNA. If this proves true for other proteins, it may be concluded that the selection pressure is less stringent on the bulk of the genome than on genes coding for polypeptides.

The human mRNA was a gift from Drs Sergio Ottolenghi and Jon Pritchard; reverse transcriptase was a gift from Dr Paul Harrison. We thank J. O. Bishop, P. Harrison, J. Paul, E. M. Southern and P. M. B. Walker for discussions. K. S. G. is a fellow of the American Cancer Society. The Beatson Institute for Cancer Research is supported by the Medical Research Council and the Cancer Research Campaign

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LETTERS TO NATURE

PHYSICAL SCIENCES

Heavy ION transfer reactions in nuclear astrophysical processes

AN appraisal of nuclear reactions in heavy ion systems which are known to be of importance in nuclear astrophysical processes indicated that reactions involving transfer of α particles could be of significant strength and hence should be included in reaction network studies which lead to the prediction of stellar abundances. The heavy ion systems of interest are $^{16}\text{O} + ^{16}\text{O}$, $^{12}\text{C} + ^{16}\text{O}$ and $^{12}\text{C} + ^{12}\text{C}$. Preliminary results from a recent experiment (T. W. C., B. W. Hooton and G. Murray, to be published) have shown in the case of the $^{12}\text{C} + ^{16}\text{O}$ system that, at energies of astrophysical importance, the channel leading to $^{20}\text{Ne} + 2\alpha$, which corresponds to the transfer on α particle, is of significant strength. This result influences predictions of the abundance of ^{20}Ne and that of elements depending on ^{20}Ne for their subsequent formation.

The purpose of this note is to identify for the heavy ion systems involved which reactions are likely to have a non-negligible rate. The astrophysical consequences of reaction channels formerly considered to be of negligible strength can only fully be appreciated when they are included in reaction networks which generate 'theoretical' abundances.

low) are also given in Table 2; these are later referred to as σ_t and have not in general been measured.

I consider here only the importance of α -transfer reactions to the exclusion of other transfer processes since the extra nuclear binding associated with such α -cluster nuclei as ^{12}C and ^{16}O ensures for this reaction the most favourable or least negative Q value of all reactions involving single or few nuclear transfer. Furthermore the nature of these nuclei ensures for the transfer a good overlap of the nuclear wavefunctions (This is particularly true for $^{12}\text{C} \rightarrow \text{Be}_{g.s.} + \alpha$ where the overlap is known to be large³).

In order to form a compound nucleus each heavy ion has to penetrate the Coulomb barrier of the other. The kinetic energy available to the ions in the upper region of astrophysical interest is comparable to the barrier height (Table 1, column 3). Under these circumstances transfer of an α particle from the one to the other heavy ion—a process which does not necessarily involve the interpenetration of the ions—is expected to be quite favourable and to become even more so as the kinetic energy of the ions is reduced to the lower region of astrophysical interest. Kozlovsky⁴ has independently considered this possibility for the $^{12}\text{C} + ^{12}\text{C}$ system. His calculations result in quite a small cross section of about 1% of σ_R for the α transfer in this case. On the other hand he indicates a very strong dependence of the transfer cross section on the binding energy of the transferred particle in the final nucleus. In the $^{16}\text{O} + ^{16}\text{O}$, $^{12}\text{C} + ^{16}\text{O}$ systems the final nucleus is ^{20}Ne in which the value of the α -binding energy is 4.73 MeV; this is much smaller than the

TABLE 1 Characteristics of α -transfer reactions in nuclear astrophysical processes

Thermo-nuclear epoch	E_0 (MeV)	Region of interest		$E_{\max}$	Energy (MeV) Coulomb barrier	Alpha transfer Reaction	Ground state Q value (MeV)	Q optimum (MeV) corresponding to ($E_{\min}$ to $E_{\max}$)
		Δ (MeV)	$E_{\min}$					
Carbon burning $^{12}\text{C} + ^{12}\text{C}$	$2.42T_9^{2/3}$	$1.05T_9^{5/6}$	1.3	8.8	≈ 6	$^{16}\text{O} + ^8\text{Be}$	-0.208	-0.14 to -0.98
Carbon-oxygen reactions $^{12}\text{C} + ^{16}\text{O}$	$3.06T_9^{2/3}$	$1.19T_9^{5/6}$	1.9	10.7	≈ 8	$^{20}\text{Ne} + ^8\text{Be}$	-2.639	-0.31 to -1.80
Oxygen burning $^{16}\text{O} + ^{16}\text{O}$	$3.90T_9^{2/3}$	$1.34T_9^{5/6}$	3.8	13.1	≈ 10.6	$^{20}\text{Ne} + ^{12}\text{C}$	-2.433	-0.24 to -0.82

At various evolutionary stages of some stars major concentrations of ^{12}C , ^{12}C and ^{16}O , and ^{16}O are believed to occur¹ in regimes corresponding to temperatures $T_9 = 1$ to 2 (and possibly up to 3.6), 1 to 3.6 and 2 to 3.6 respectively (where T_9 is in units of 10^9 K). The lower and upper limits in each case correspond generally to temperatures associated with hydrostatic and explosive burning stages respectively. Table 1 shows the most effective range of energies $E_{\min}$ to $E_{\max}$ (calculated in terms of E_0 and Δ as defined in ref. 2) promoting nuclear reactions under these conditions.

In Table 2 the reaction channels measured for the $^{12}\text{C} + ^{12}\text{C}$, $^{12}\text{C} + ^{16}\text{O}$ and $^{16}\text{O} + ^{16}\text{O}$ systems are listed. These correspond to reaction channels expected to be most important from statistical evaporation theory following the formation of a compound nucleus. The sum of the principal components of the cross section is later called σ_R . The α -transfer reaction and equivalent three body breakup process (discussed be-

value of 7.16 MeV for ^{16}O , the final nucleus in the case of $^{12}\text{C} + ^{12}\text{C}$. We thus expect significantly higher cross sections for transfer in the $^{16}\text{O} + ^{16}\text{O}$ and $^{12}\text{C} + ^{16}\text{O}$ systems. A recent calculation (P. J. A. Buttle, private communication) for the $^{12}\text{C} + ^{16}\text{O}$ system showed that the cross section for the α transfer $^{12}\text{C}(^{16}\text{O}, ^{20}\text{Ne})^8\text{Be}$ to the ground state of ^{20}Ne alone could be as high as 12 mb at 10 MeV. This is about 2-3% of σ_R . Recent theoretical estimates thus indicate that this reaction channel is of some significance in astrophysical processes.

The crux of the matter lies in the degree to which the cross section decreases as the bombarding energy (or temperature) decreases. It is at least conceivable that the latter falls less rapidly than σ_R and that over the region of astrophysical interest it makes an even more significant contribution to the overall reaction rate than the value of 2 to 3% quoted above would suggest, and, if so, should be included

in reaction network studies from which abundances are calculated.

Alpha transfer in the three systems considered is also of considerable interest from the point of view of the dynamics

TABLE 2 Components of σ_R and σ_t

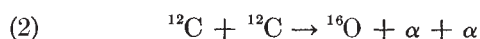
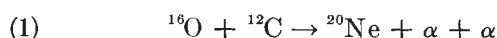
	Principal compound channels	α transfer channel and equivalent three-body process
$^{12}\text{C} + ^{12}\text{C}$	$^{20}\text{Ne} + \alpha$ $^{23}\text{Na} + \text{p}$	$^{16}\text{O} + ^8\text{Be}$ $^{16}\text{O} + \alpha + \alpha$
$^{12}\text{C} + ^{16}\text{O}$	$^{24}\text{Mg} + \alpha$ $^{27}\text{Al} + \text{p}$	$^{20}\text{Ne} + ^8\text{Be}$ $^{20}\text{Ne} + \alpha + \alpha$
$^{16}\text{O} + ^{16}\text{O}$	$^{28}\text{Si} + \alpha$ $^{31}\text{P} + \text{p}$	$^{20}\text{Ne} + ^{12}\text{C}$

of heavy ion interactions. It has been predicted and demonstrated⁵ that for heavy ion reactions those reaction channels in which the distance of closest approach in the ingoing and outgoing channels is similar are favoured. The effect of this is to define a region of reaction Q values (Q -value window) around a value Q_{optimum} given by

$$Q_{\text{opt}} \approx ((z_f Z_f / z_i Z_i) - 1) E_{\text{CM}}$$

in which reactions are favoured. z_f, Z_f are the atomic numbers of the heavy ions in the outgoing channel and z_i, Z_i refer to the ingoing channel. The last column of Table 1 indicates that at the upper end of the energy range of astrophysical interest the α -transfer reactions to the ground states of the final nuclei in all three systems should be favoured.

Although the discussion above has emphasised the possible importance of α transfer in the heavy ion systems of astrophysical interest it should also be mentioned that, in two of the systems considered, three-body breakup, namely



leads to the same final nucleus as that involved in α transfer. No direct measurements of this contribution to the total cross section in the cases discussed seem to have been made although it is the sum of this process and α transfer which, for example, determines the amount of ^{20}Ne produced in $^{16}\text{O} + ^{12}\text{C}$ interactions and which modifies the number of α particles available for further interactions.

Of the three systems considered: oxygen-burning at temperatures characteristic of explosive nucleosynthesis has been shown⁶ to produce the observed abundances of the nuclei between ^{28}Si and ^{42}Ca and carbon-burning regulates the amount of ^{16}O and ^{20}Ne available for late evolutionary stages; the importance of $^{12}\text{C} + ^{16}\text{O}$ interactions during explosive nucleosynthesis has recently been dealt with by Woosley, Arnett and Clayton¹. A literature search revealed no direct measurement of α -transfer cross sections for any of the reactions detailed above in the energy regions on or below the Coulomb barrier although an estimate has been made at two energies close to the barrier in the $^{16}\text{O} + ^{16}\text{O}$ case⁷. This lack of data reflects the difficulty of detecting and identifying heavy ion species in the energy range close to 0.5 MeV per nucleon with conventional solid state detector telescope systems. A good case exists for a systematic study of all three reactions and their energy dependence not only for its astrophysical importance but also to provide measurements to clarify aspects of the heavy ion interaction.

This suggests that the reaction channels leading to ^{20}Ne which were discussed above should be included in calculations of the stellar ^{20}Ne abundance and related elemental

abundances.

It is planned to extend the measurements as a function of angle and energy to compare the total yields of ^{20}Ne and ^{24}Mg . The measured yield of ^{24}Mg will provide a useful check on other method of measuring what is the principal contribution to σ_R . The systems $^{16}\text{O} + ^{16}\text{O}$ and $^{12}\text{C} + ^{12}\text{C}$ are also being studied.

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Association between the mid-winter stratospheric circulation and the first observation in the following summer of noctilucent clouds

NOCTILUCENT clouds (NLCs) are seen mainly in the summer months at high latitudes over both hemispheres. They appear in a thin layer at a height of about 82 km in the mesopause region where temperatures are the coldest in the Earth's atmosphere. A temperature of 150 K or less at the mesopause has been shown, by rocket soundings in the presence of NLCs, to be a necessary condition for the existence of these clouds¹. Rocket sampling experiments and ground-based observations indicate that NLC particles probably consist of a volatile substance, believed to be ice, coated on volatile nuclei that may be hydrated ions^{2,3}.

Thus two important variables, which help to determine whether the formation of NLCs will occur, are the water vapour content and temperature. Both parameters depend on the mesospheric circulation, whose main features at a particular latitude vary seasonally. In consequence it has been suggested that observations of NLCs might be used as a guide to the mesospheric circulation. For example, the onset of NLCs in early summer might relate to the development of the summertime circulation pattern in the mesosphere.

The first observations of NLCs each year fall into three categories:

(a) Mid-winter, for example January 5, 1964 at Lerwick, Scotland⁴. There are few of these observations.

(b) Spring equinox. Occasional reports during March and April have been noted. However they are confined to data from North America and the USSR.

(c) Early summer. Western European data regularly start in late May or early June.

Observations in category (a) have been shown to be correlated with record temperature and wind observations in the lower stratosphere⁵. Although it is generally assumed that the wintertime mesopause is always much warmer than in sum-

mer, there have been observations by the grenade technique in mid-winter of mesopause temperatures as low as 148 K⁶. These were also associated with similar extreme disturbances in the stratosphere. On such occasions temperature changes of opposite sign appear to take place at adjacent levels. Thus the mesosphere may cool to below mean summertime temperatures while the stratosphere is undergoing a 'warming.' These anomalous mid-winter phenomena are temporary, lasting perhaps for a few days, before the circulation starts reverting to the normal wintertime pattern.

Category (b) observations are difficult to explain and are not discussed in this paper. It is hard to understand why there should be no equinoctial observations for Western Europe if those from the USSR and North America are valid.

The observations in category (c) are of particular interest. They are from a series of papers published in the *Meteorological Magazine* by Mr J. Paton of the University of Edinburgh⁷. The data have been subject to strict criteria before acceptance and have been recorded by an experienced group of observers. As the observations are limited to Western Europe between latitudes 50° and 60° N, any variations due to latitudinal or longitudinal differences are greatly diminished. These data demonstrate a surprising feature which is shown in Fig. 1. This is the tendency for the first observation of NLCs to be earlier in those summers following a major stratospheric warming (MSW) in the previous winter. The disturbance to the mid-winter stratospheric circulation is

represented in the figure by the maximum altitude (in km) reached at the North Pole by the 10 mbar surface during the months of December and January. These data are taken from the reports published by the Staff of the Stratospheric Research Group at the Free University of Berlin. The 10 mbar surface rose to heights ≥ 30.80 km only for the seven years when an MSW occurred in mid-winter (1960, 1963, 1966, 1968, 1970, 1971, 1973). In each case a reversal of the latitudinal temperature gradient at 10 mbar took place (warm air replaced cold air over the pole) and the westerly circulation collapsed to be replaced, for at least a few days, by a weak easterly flow.

In six out of these seven years, the first NLC observation was never later than June 2. The exception is 1963 when the first observation was on June 28. This datum point is excluded from Fig. 1 on the following grounds:

(a) The observers in 1963 were not as numerous or as familiar with the clouds as today—regular observations to see whether NLCs were present did not start until the IQSY in 1964.

(b) A big problem in late May and June 1963 was the poor visibility. Much cloud and rain were reported by the relevant meteorological stations at that time. Leuchars, one of the most likely NLC reporting stations, recorded twenty-six cloudy nights in June. A doubtful observation of NLCs mixed with cirrus and patchy hill fog was made on May 29—this was rejected for publication.

For the six years of data for which an MSW occurred during the preceding winter, the mean date of the first NLC observation was May 28 with a standard deviation of only 2.7 d. For illustration, the least-squares technique was used to fit to the data a straight line

$$y = (19.6 \pm 3.7) - (5.3 \pm 1.6)x$$

where y is the date of the first NLC observation in days after May 25 and x is the maximum height of the 10 mbar surface in km above 28 km.

These results are surprising. It was not expected that such a clear association would be apparent between events occurring four to six months apart and at different levels of the upper atmosphere. Following on from these results it was thought that there might be an association between the stratospheric circulation at the final spring warming when the changeover from winter to summer conditions occurs and the first reports of NLCs. Until 1964 it was possible to classify these final warmings at 10 mbar into early or late types; however, no early final warmings occurred after 1964 until 1972 (ref. 9). Further, the mean monthly 10 mbar heights at the pole for the months of May were found to show no correlation with the presence the previous winter of an MSW or the first reports of NLCs.

Thus in the lower stratosphere the effects of an MSW seem to be smoothed out by the end of March whereas at the mesopause, effects are still visible at the end of May. These results emphasise the importance of studying the dynamic interactions between the stratosphere and mesosphere and of considering the phenomenon of a stratospheric warming as affecting the mesosphere as well as the stratosphere.

I acknowledge the support of a Royal Society travel grant for a visit to the Meteorological Institute, Free University of Berlin in May 1973. I thank Professor K. Labitske for discussions during my visit to Berlin and Mrs M. Hallissey of the Balfour Stewart Auroral Laboratory, University of Edinburgh for providing NLC observational data.

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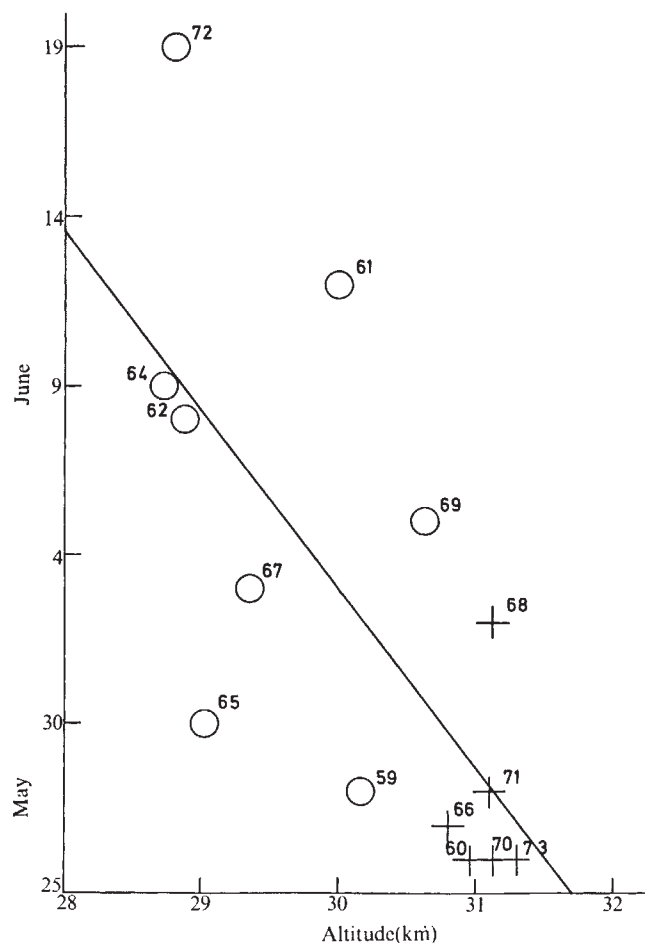


FIG. 1 Comparison of the date of the first NLC observation with the maximum height (km) reached at the North Pole by the 10 mbar surface during the months of December and January the preceding winter. ○, Year without MSW; +, year with MSW.

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Electric cloud and weather modification with intense relativistic electron beams

THE problems confronting weather modification by seeding methods are well known and centre on the dispersion of the seeding agent over a large air volume. In a different method for artificial cloud modification proposed by Vonnegut and Moore¹ electric charges are released by corona discharges into updraughts from the ground by a system of wires at a high electric potential. In this method the electric charges are much more rapidly dispersed, due to a rather large ion mobility in a thundercloud electric field, than is otherwise possible for seeding agents dispersed by turbulent convection. But the system of ground wires seems to be impractical for a weather modification technique with the demand for high mobility.

I therefore propose here an alternative method of electric cloud and weather modification based on the projection of a rapid sequence of intense relativistic electron beams into the atmosphere. The maximum range in the atmosphere of relativistic electrons is given by the radiation length $l_0 \sim 280$ m and which is rapidly approached for electron energies in excess of 50 MeV. A beam of electrons would normally disperse rapidly in the radial direction due to multiple scattering. This however, is not the case for an intense beam of relativistic electrons which is confined by its own magnetic field. Beams up to 10^6 A and with electron energies of many million electron volts lasting up to 100 ns have been already produced by the combination of a Marx surge generator with a Blumlein transmission line, connected to a field emission diode. The total beam energies so far achieved reach the value of megajoules. If such a beam is projected into the atmosphere it will not disperse radially but rather will produce a channel of hot air, with a maximum channel length equal to the radiation length in air. If now a second beam is projected into this channel after a time lapse which is equal to the time for the air to expand in the channel heated by the first beam and which is given by $\tau_{exp} \sim r/a$, where r is the beam radius and a the velocity of sound in the heated air, then the second beam will in part propagate through air of reduced density due to the thermal expansion caused by the first beam. If for example, the first beam heats the channel up to $\sim 10^4$ K the velocity of sound would be $\sim 10^5$ cm s⁻¹, and if the original beam radius is $r \sim 0.1$ cm the time for expansion would be $\tau_{exp} \sim 10^{-6}$ s. Because of the reduced attenuation the second beam will propagate further than the first beam. A third beam will propagate even farther, and so on. A sequence of beams rapidly projected along the same path will therefore tunnel into the atmosphere much deeper than otherwise possible with only

one beam. Such a rapid sequence of beams can be obtained by a parallel arrangement of Marx generators to be discharged in a programmed sequence by triggered spark gap switches over the same Blumlein line and discharge diode.

I define the ratio $T_0/T = \kappa$ where T_0 is the air temperature under normal conditions and T the temperature in the channel heated by the beam. For $T \sim 10^4$ K, $\kappa \sim 3 \times 10^{-2}$. With a beam channel volume of $\pi r^2 l_0 \sim 10^3$ cm³ a beam energy $\epsilon \sim 10^4$ J would raise the temperature to $T \sim 10^4$ K. The first beam pulse will penetrate the distance $\delta_1 = l_0$. The second beam will add to this the penetration length $\delta_2 = (1 - \kappa)l_0$, the third will add $\delta_3 = (1 - \kappa)\delta_2 = (1 - \kappa)^2 l_0$, and the n th beam will add $\delta_n = (1 - \kappa)\delta_{n-1} = (1 - \kappa)^{n-1} l_0$. The total penetration length is therefore given by

$$l_n = \sum_{n=1}^n \delta_n = l_0 \sum_{n=1}^n (1 - \kappa)^{n-1} = [1 - (1 - \kappa)^n] l_0 / \kappa.$$

For $n \rightarrow \infty$, $l_\infty = l_0 / \kappa$. For $l_0 = 2.8 \times 10^4$ cm and $\kappa = 3 \times 10^{-2}$, $l_\infty \sim 10$ km. For $n = 10$ pulses the length would be $l_{10} = 2.4$ km. In this case each beam pulse delivers $\sim 10^4$ J such that the total energy delivered in ten subsequent pulses, with a time lapse of $\sim 10^{-6}$ s between the pulses, is $\sim 10^5$ J. The total time to discharge ten subsequent pulses is $\sim 10 \times 10^{-6}$ s = 10^{-5} s and is short enough to prevent the beam channel from being destroyed by either heat conduction or wind motion. A capacitor bank of 10^5 J has an approximate weight of several tons. The capacitor bank could be thus mounted on a heavy truck or airplane and charged up by a combustion engine, making the electron beam machine mobile, a desirable requirement if the beam is to be employed for cloud or weather modification.

This method promises the following: (1) It permits the disposal of a large negative electric charge into a chosen volume of the atmosphere or of a cloud. (2) The intense relativistic electrons release within the atmosphere a large X-ray flash producing many pairs of positive and negative ions. (3) The beams create within the atmosphere channels of high electrical conductivity.

The implications of these effects to potential cloud and weather modification are manifold. The most important ones seem to be: (1) The local release of electric charges can result in greatly increased electric fields within a certain cloud region leading to enhanced cloud droplet coalescence resulting in increased precipitation. (2) The release of electric charges in an updraught may accelerate the thundercloud electrification by the Vonnegut-feedback mechanism² and conceivably in this way also increase precipitation. (3) The creation of a large number of ion pairs will increase the electric conductivity of the air which could be used for lightning suppression. (4) The creation of long channels of high electrical conductivity could be used to trigger artificially cloud-cloud or cloud-ground discharges.

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Astrophysical fuel-coolant interactions

If a hot liquid comes into contact with a cold vaporisable liquid a violent explosion may occur¹⁻⁵; such interactions are often called fuel-coolant interactions (FCIs). In this letter we apply FCI theory to the problem of matter and antimatter interacting on an astronomical scale.

Buchanan and Dullforce⁶ explain FCIs as follows: (1) an initial perturbation causes a small vapour bubble to form at the fluid interface; (2) as the vapour condenses the bubble collapses and, because of local asymmetry, a jet of cold liquid forms; (3) the jet penetrates the hot fluid and disintegrates; and simultaneously (4) the jet material is heated; (5) as the jet is vaporised a new bubble forms and expands. This process continues cyclically through stages (2)–(5). D. J. Buchanan (to be published) has shown that the initial perturbation is damped out if the external pressure is greater than some threshold value, and recently we⁷ have used this fact to suggest that volcanic hydroexplosions⁸ in the form of FCIs⁹ do not occur at sea depths greater than some critical value.

Similar arguments can be applied to the problem of interacting clouds of matter and antimatter by noting that the FCI model can be generalised to describe a fluid interface instability of wider application. Conditions necessary for this instability are an energy releasing reaction (for example through the contact of hot and cold liquids), the production of an intermediate phase which is then condensed (for example the vapour bubble), and an asymmetry which causes intermixing by jet penetration and thus further energy releasing reactions. The energy releasing reaction need not be thermal and the intermediate phase need not be a vapour; for example, the energy releasing mechanism might be chemical.

Alfvén¹⁰ has suggested that regions of matter and antimatter could be separated by a thin Leidenfrost layer of radiation sustained^{11,12} by gradual annihilation. The following instability can be postulated. A perturbation locally decreases the thickness of the Leidenfrost layer, thus the annihilation rate increases and a radiation bubble forms. Initially the bubble expands and, provided the radiation escapes, it then collapses. Departure from mirror symmetry can result in the formation of a jet of matter, say, if the bubble is in the matter region; if this jet penetrates the antimatter region further annihilation will occur and another bubble will form. Significant differences in, for example, density, viscosity or magnetic field strength constitute departures from symmetry. Thus the possibility exists of explosive mixing of matter with antimatter analogous to FCIs.

If the ambient pressure at the interface is greater than some threshold value, P_{th} , then the initial perturbation is damped out. According to Buchanan (to be published)

$$P_{th} = (3\alpha^3)/1 + \alpha + \alpha^2 P_i \quad (1)$$

where P_i is the pressure in a bubble at its formation in any cycle and α is given by

$$\alpha^3 = (3/16)\beta d_c^2 L_c \quad (2)$$

Here β is the fraction of the jet that is annihilated and d_c and L_c are dimensionless non-negative numbers less than unity which characterise the jet^{6,13} and are determined by the degree of local asymmetry. For perfect symmetry d_c and L_c are both zero.

If ρ is the density of a jet of volume V then the energy released by annihilation is $2\rho c^2 V$ giving an initial pressure of $(4/3)\rho c^2$ and so for $\beta = 1$

$$P_{th} \simeq \frac{3}{4} d_c^2 L_c \rho c^2 \quad (3)$$

If the ambient pressure is greater than P_{th} the instability is suppressed.

As an illustration of the consequences of equation (3) consider a self-gravitating sphere of mass M and radius R with an antimatter core (or *vice versa*). The central pressure P_c and the mass are given by¹⁴

$$P_c = W_n M^2 G / R^4 \quad (4)$$

and

$$M = C_n \frac{4}{3} \pi R^3 \rho \quad (5)$$

where ρ is the central density, G is the gravitational constant, and W_n and C_n are dimensionless numbers depending on the polytropic constant. From equations (3), (4) and (5) we deduce that the matter/antimatter interface is stable to an 'intermediate phase instability' if the Schwarzschild number $S (= 2GM/Rc^2)$ satisfies

$$S > (9/8)(d_c^2 L_c / \pi W_n C_n). \quad (6)$$

If the adiabatic constant $\gamma = 5/3$ then $n = 3/2$ and $W_{3/2} C_{3/2} = 0.129$ and for $\gamma = 4/3$, $n = 3$ and $W_3 C_3 = 0.213$. Hence $W_n C_n$ is of order 10^{-1} for likely values of γ . The largest likely values of d_c and L_c are those appropriate to bubble collapse adjacent to a solid wall,¹³ namely about $1/4$ and $1/2$ respectively. Thus the inequality (6) becomes

$$S \gtrsim 5 \times 10^{-2} \quad (7)$$

This value of the Schwarzschild number is large compared with those normally encountered in astrophysics; for example, both the Sun and the galaxy have $S \simeq 10^{-6}$. If the radius of the Sun were 70 km, like that of a neutron star, then condition (7) would just about be satisfied. When condition (7) is satisfied we have the interesting possibility of a region of antimatter existing at the centre of a large region of matter, the whole system being stable against dynamic mixing arising from this type of instability.

Other examples, such as the collision of two bodies under gravitational attraction or through their random velocities, result in a similar condition involving the Schwarzschild number, but in these cases the high pressure generated on impact is only a transient feature, and cannot permanently inhibit the instability.

The immediate implication of this admittedly crude model is that the stability of the Leidenfrost layer postulated by Alfvén depends critically on the Schwarzschild number and the degree of symmetry at the matter/antimatter interface.

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Atmospheric carbon dioxide levels as indicated by the stable isotope record in wood

THE concentration of CO₂ in the atmosphere has increased from about 290 parts per million (p.p.m.) by volume in 1900 (ref. 1) to 323 p.p.m. in 1972 (ref. 2) and this increase is generally held to be due chiefly to combustion of fossil fuels. Indeed by 1970 this artificial mechanism had introduced to the atmosphere an amount of CO₂ equivalent to 22% of the natural or pre-industrial atmospheric inventory³. About half of the added CO₂ seems to have been transferred to the other major reservoirs of the dynamic carbon cycle, the oceans and biosphere. Before examining the threat to global climate and marine life⁴, future CO₂ levels from this source must be predicted. Before such extrapolations can be confidently applied, however, we must fully understand the past response of the atmosphere to fossil CO₂ emission. Since direct measurements of atmospheric CO₂ are either not available or considered unreliable indirect methods of assessing the CO₂ buildup must be invoked. Here a novel and potentially valuable approach is presented.

The carbon isotopic composition of fossil CO₂ can be distinguished from that of natural atmospheric CO₂ in two significant ways; (1) the radioactive isotope of carbon, ¹⁴C, present as one part per 10¹² in atmospheric carbon, is totally absent in fossil carbon because of the rapid radioactive decay of ¹⁴C (half-life 5,730 yr) relative to the typical age of fuel deposits; (2) the secondary stable isotope of carbon, ¹³C, on average about 1.1% of nature's total carbon, is 2% less abundant in terrestrial plants and their fossil remains (coal, petroleum, natural gas) than in atmospheric CO₂ (ref. 5; Fig. 1). The burning of fossil fuels, therefore, has released to the atmosphere increasing amounts of both ¹⁴C and ¹³C-depleted CO₂. It seems reasonable to suppose that both the ¹⁴C/¹²C and ¹³C/¹²C ratios of atmospheric CO₂ will have decreased in sympathy with the addition of lighter CO₂. Furthermore, such trends should be permanently recorded in the annual tree rings of wood which, in effect, derive their carbon directly from the ambient atmosphere. Indeed the decline of the ¹⁴C/¹²C ratio is well documented. Extrapolation from ¹⁴C data to levels of fossil CO₂ is, however, of limited value since there is evidence that natural variations in ¹⁴C/¹²C have accompanied the artificial dilution effect⁶. Until now there has been no attempt to evaluate the stable isotope record. If isotopic fractionation during photosynthetic assimilation of atmospheric CO₂ by growing wood has remained constant, ¹³C/¹²C assay of tree rings of known age can in theory yield additional information on the past levels and isotopic composition of atmospheric CO₂.

We have measured the ¹³C/¹²C ratios of individual rings from two trees: (1) an oak (*Quercus robur*) planted 1848, felled 1968, from the Forest of Dean (51°48'N, 2°37'W), England and (2) a European larch (*Larix decidua*) planted 1892, felled 1972, from Glengarry Forest (57°01'N, 4°51'W), Scotland. To minimise isotopic fractionation effects during experimentation, the wood samples were not subjected to any chemical treatment prior to conversion to CO₂ for isotope ratio measurement. The ¹³C/¹²C ratios were determined using a double collecting, 90° deflection, 6 cm radius Micromass 602B mass spectrometer and the results expressed to a precision of ±0.1‰ (2σ) relative to the primary standard, PDB belemnite limestone. On this scale the isotope

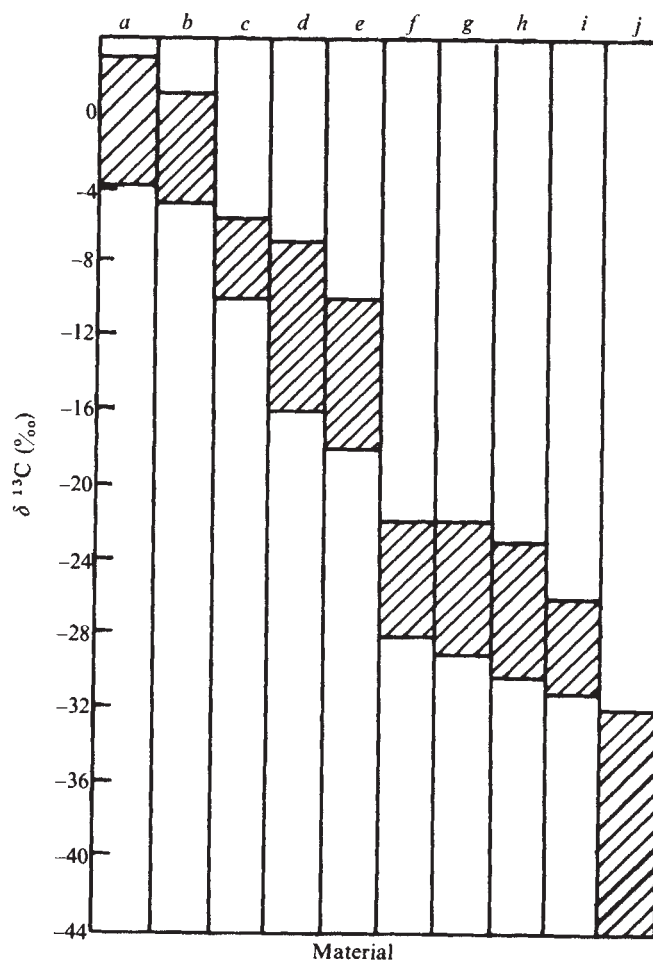


FIG. 1 Carbon isotope ratios of various carbonaceous materials. a, Carbonates; b, volcanic CO₂; c, atmospheric CO₂; d, marine plants; e, marine organisms; f, coal; g, land plants; h, liquid hydrocarbons; i, organic sediments; j, natural gas.

data are quoted as δ¹³C, the per mille deviation of sample ¹³C/¹²C from that of the standard, by the expression⁷

$$\delta^{13}\text{C}_{(\text{sample})} = \frac{^{13}\text{C}/^{12}\text{C}_{(\text{sample})} - ^{13}\text{C}/^{12}\text{C}_{(\text{standard})}}{^{13}\text{C}/^{12}\text{C}_{(\text{standard})}} \times 1,000\text{‰}.$$

To smooth the distribution of results a 10-yr running mean was calculated for each series and the two curves plotted as shown in Fig. 2. Before detailed discussion of these results it should be noted that the systematic difference (~1.1‰) between the early sections of both curves can reasonably be attributed to a smaller fractionation effect in larch wood synthesis.

Both trees show a rapid decline in δ¹³C during the early years of this century amounting to 1.5‰ from 1900–20 for the oak and 1.1‰ from 1905–21 for the larch (1.2‰ for the oak). The two curves also exhibit similar trends after 1944, rising to a more positive δ¹³C by 1958 and then declining rapidly to 1964. Between 1922 and 1944 the curves are dissimilar.

Assuming constant CO₂ fractionation factors of (1) -19.9‰ and (2) -21.0‰ between the atmosphere and (1) larch and (2) oak respectively⁸ there has been an average decline in atmospheric δ¹³C of 1.7‰ from -4.9‰ in 1900 to -6.6‰ in 1964. If we consider the 1964 CO₂ concentration of 316 p.p.m.⁹ (δ¹³C₁₉₆₄ = -6.6‰) as the result of a combination of the 1900 CO₂ concentration (δ¹³C₁₉₀₀ =

-4.9‰) and additional fossil CO₂ ($\delta^{13}\text{C}_f = -26.0\text{‰}$) then the 1900 CO₂ concentration can be calculated from

$$[\text{CO}_2]_{1900} = \frac{[\text{CO}_2]_{1964} (\delta^{13}\text{C}_{1964} - \delta^{13}\text{C}_f)}{(\delta^{13}\text{C}_{1900} - \delta^{13}\text{C}_f)}$$

$$= 290.5 \text{ p.p.m.}$$

This figure is in excellent agreement with the generally accepted estimate of 290 p.p.m.¹ Although this ideal consistency of the model may be coincidental, similar calculations are especially instructive for the earlier years of this century.

Both trees show a decline in $\delta^{13}\text{C}$ of 1.5‰ between 1900 and 1920. Such a decrease could not have been caused even by complete retention of all the fossil CO₂ introduced to the atmosphere from 1900-20 (ref. 10) (a CO₂ input equivalent to 7.9 p.p.m.); that is

$$\delta^{13}\text{C}_{1920} = \frac{\delta^{13}\text{C}_{1900}[\text{CO}_2]_{1900} + \delta^{13}\text{C}_f([\text{CO}_2]_{1920} - [\text{CO}_2]_{1900})}{[\text{CO}_2]_{1920}}$$

$$= -5.5\text{‰} \text{ (a decrease of only } 0.6\text{‰)}$$

A 1.5‰ decline due to an addition of CO₂ of $\delta^{13}\text{C} = -26.0\text{‰}$ corresponds to a calculated $[\text{CO}_2]_{1920}$ concentration of 312.7 p.p.m., an increase of 22.2 p.p.m. over the 1900 figure of 290.5 p.p.m. as compared to a total fossil CO₂ addition of 7.9 p.p.m. Intriguingly, this figure of 312.7 p.p.m. is fair agreement with some early and hitherto disputed direct measurements of atmospheric CO₂ concentrations which nevertheless were considered sufficiently reliable to be included by Callendar¹ in his summary of twentieth century levels. The implication therefore is that, during the early decades of this century, there was an appreciable additional source of CO₂ of $\delta^{13}\text{C}$ similar to that of land plants (-25 to -26‰). The possibility of a CO₂ contribution from volcanic emission or from warming of the oceans ($0 \leq \delta^{13}\text{C} \leq -5\text{‰}$) is apparently excluded. A likely source of this additional CO₂ could have been the oxidation of organic litter of terrestrial land plant composition accompanying enlargement of the world's cultivated farmland at the expense of forests and grassland. In support of this premise is the estimate by Revelle¹¹ that the observed increase in arable land of $\sim 1.6 \times 10^6$ million square miles corresponds to a humic CO₂ input of $\sim 10^{17}$ g CO₂, a figure of exactly the required order of magnitude.

It is evident that the rapid increase in CO₂ inferred above did not continue until the present. During the period 1944 to 1958 in particular both trees record trends to more

positive $\delta^{13}\text{C}$. It is likely then that during recent decades an additional influence has become dominant and that, through partial compensation of the fossil CO₂ flux and resultant $\delta^{13}\text{C}$ levels, it has stabilised the atmospheric CO₂ burden. The most probable causes of this latter effect are enhanced CO₂ exchange with and uptake by the oceans, perhaps in sympathy with the observed 0.1-0.2° C drop in mean global temperatures since 1940 (ref. 12), or alternatively an increase in the size of the terrestrial biomass in response to the rise in atmospheric CO₂ levels. Whatever the responsible mechanism it is apparent that an important stabilising influence has been exerted. The discrepancies between the curves for the 1922-44 interval may serve to illustrate the dangers of extrapolation from results which could be subject to local effects. It is nevertheless possible that the data from this period do indeed reflect local differences in response to the transition between two competing mechanisms. During the past 15 yr the decline in $\delta^{13}\text{C}$ is in line with the reported direct measurements of the increase in atmospheric CO₂.

The assumption of constancy of fractionation between atmospheric CO₂ and growing wood during the past 70 yr may perhaps be open to doubt. Craig¹³, however, has reported that the long-term trend of wood $\delta^{13}\text{C}$ from 900 BC to 1600 AD was constant to within 1‰. Although minor variations could be induced by the effects of varying external conditions on the assimilation and respiratory process of a tree, it seems most improbable that a 2‰ decrease in wood $\delta^{13}\text{C}$ could be attributed to such factors particularly in view of the considerable magnitude of the fossil CO₂ injection considered here. Furthermore, it is of interest that Dequasie and Grey⁸ found a similar rapid 1.3‰ decrease in United States poplar wood $\delta^{13}\text{C}$ from 1912-27 and an overall decrease of 2.3‰ by 1966.

While more data are required for a precise assessment, the results of this preliminary study imply (1) an additional source of CO₂ of $\delta^{13}\text{C} \sim -26\text{‰}$, in conjunction with fossil fuel combustion, contributed to rapidly increasing atmospheric CO₂ concentrations during the first 20-30 yr of this century and (2) variations in exchange and removal rates of CO₂ across the respective atmosphere/biosphere/ocean interfaces have occurred in view of the irregular and somewhat unexpected trends of $\delta^{13}\text{C}$ during the past 40 yr. Therefore, in view of the apparent complexity of the interactions and responses involved, prediction of future CO₂ levels probably cannot be performed with confidence. But continued research along these lines should assist towards the evaluation of the controlling geophysical and geochemical parameters within the carbon cycle and eventually towards a more definite assessment of the likely fate of fossil CO₂.

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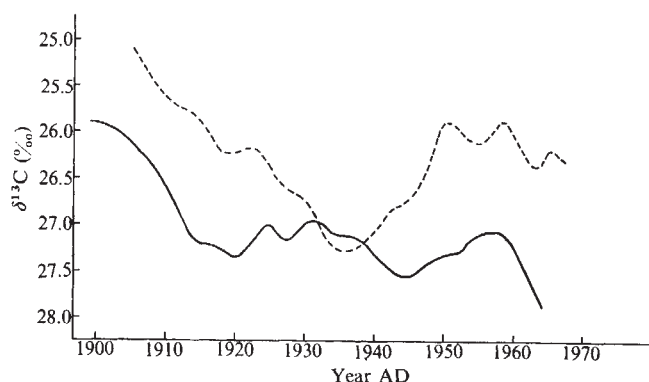


FIG. 2 Temporal variations of carbon isotope ratio observed in twentieth century larch (----) and oak (—) wood.

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BIOLOGICAL SCIENCES

Recovery of Yeast from Transient Inhibition of DNA Synthesis

IN the simple eukaryotic cell *Saccharomyces cerevisiae* nuclear division, cell division and the start of new budding cycles depend on DNA synthesis¹. Protein synthesis is required for the recovery of new budding and cell division after transient exposure to hydroxyurea, a specific reversible inhibitor of DNA synthesis². In this report I have attempted to answer two questions. (1) Does recovery of nuclear division require protein synthesis? (2) Does recovery of DNA synthesis itself require protein synthesis?

To answer the first question a log phase culture was exposed to hydroxyurea to arrest cells with undivided nuclei in the necks between mother cells and buds. In a portion of the culture from which hydroxyurea was removed and replaced by an inhibitor of protein synthesis, anisomycin, the nuclei did not divide (Fig. 1). Therefore, protein synthesis is needed for the recovery of nuclear division. In conjunction with the previous findings² this indicates that during exposure to hydroxyurea some of the proteins needed for the execution of all events dependent on DNA synthesis are not made (or do not remain stable).

When protein synthesis is blocked after exposure to hydroxyurea nuclear division is prevented, but the complete round of DNA synthesis required for nuclear division might not be prevented. To test this possibility a log phase culture was exposed to hydroxyurea to yield cells with nuclei in the neck. Protein synthesis was blocked with anisomycin, and hydroxyurea was removed. After 1.5 h, hydroxyurea was reintroduced to part of the culture (to prevent further DNA synthesis), anisomycin was removed from the whole culture and nuclear division was monitored (Fig. 2). If the DNA synthesis required for nuclear division occurred in the presence of anisomycin, then reintroduction of hydroxyurea when the antibiotic was removed would not prevent subsequent nuclear division. I found that when the anisomycin was removed, the nuclei divided with the same kinetics whether or not hydroxyurea was present. The result was the same with initial exposures of 2.5 and 3.5 h to hydroxyurea and when the concentration of anisomycin was 10^{-4} M (three times the amount completely inhibiting protein synthesis more than 90% within 5 min) or 3×10^{-4} M. I therefore conclude that blocking protein synthesis when hydroxyurea is removed does not prevent a full round of DNA synthesis.

This conclusion was confirmed by experiments in which DNA synthesis was followed by measuring the incorporation

of radioactive precursors. On six occasions portions of a steady state labelled log phase culture were exposed to anisomycin or first transiently exposed to hydroxyurea and then exposed to anisomycin. Exposure times to hydroxyurea varied from 2 to 3.5 h and the concentration of anisomycin varied from 10^{-4} M to 3×10^{-4} M. The residual increase of DNA in the presence of anisomycin was measured as described in the legend of Fig. 3 (my unpublished results). For cultures pretreated with hydroxyurea the average increase was 85%: this was the value predicted, since 33% of the cells were engaged in DNA synthesis when hydroxyurea was added². On average, the genomes of these cells were half replicated, which accounts for about 17% of the DNA in the culture. Since this DNA did not replicate again the expected value was $100\% - 17\%$ or 83%. By the same reasoning a 17% increase was expected for cells not previously exposed to hydroxyurea. The value found was 24%, which was high. This may be due to continued mitochondrial DNA synthesis and the experimental error inherent in measuring DNA in yeast.

Special proteins must be made for the initiation of DNA synthesis. They are made and remain functional during exposure to hydroxyurea, as demonstrated by the following observation. When protein synthesis was inhibited with anisomycin before the start of DNA synthesis in synchronised cells, initiation of DNA synthesis was prevented. If, however, DNA synthesis was first prevented by hydroxyurea, and anisomycin was added when hydroxyurea was removed, DNA was synthesised in the absence of protein synthesis (Fig. 3).

In the normal yeast cell cycle protein synthesis is required for initiation but not continuation of DNA synthesis^{3,4}. My results show that during a 3.5 h exposure to hydroxyurea,

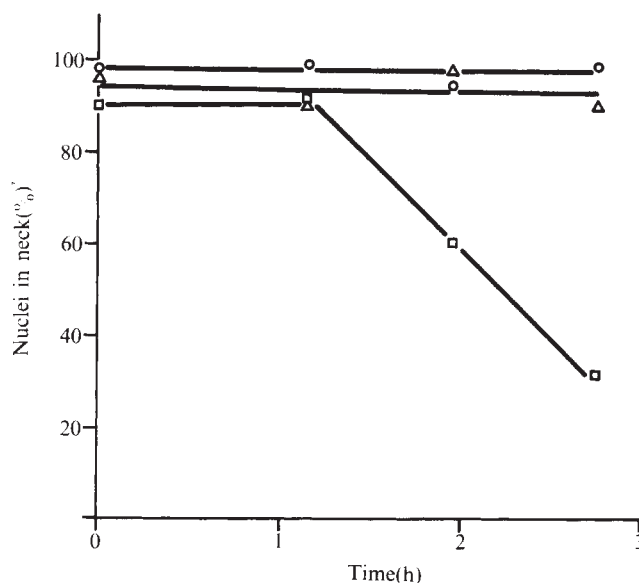


Fig. 1 Effect on nuclear division of adding anisomycin after reversible inhibition with hydroxyurea. For all experiments the yeast strain and growth medium were as described previously². Unless otherwise stated, the concentrations of hydroxyurea and anisomycin were 0.075 M and 3×10^{-4} M, respectively. For all medium changes, cells were collected on filters², washed with 3 volumes of prewarmed medium and resuspended in prewarmed medium. The same medium was used for washing and resuspension. Hydroxyurea was added to a log phase culture. After a 3.5 h incubation (time 0 on the graph) cells were transferred to fresh growth medium (□) or to medium containing anisomycin (Δ), while incubation in the presence of hydroxyurea was continued for a third sample (○). Duplicate 5 ml portions were removed for nuclear staining¹⁵.

the proteins for DNA synthesis are made, remain functional, and can be expressed in a full round of DNA synthesis in

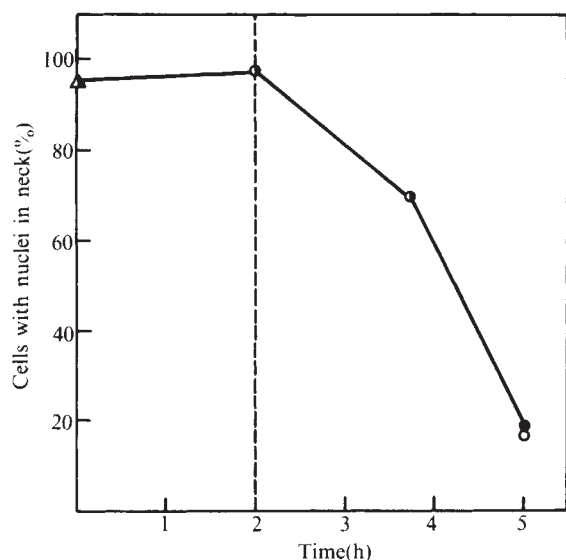


Fig. 2 Effect on nuclear division of successive exposures to hydroxyurea, anisomycin and hydroxyurea. Hydroxyurea was added to a log phase culture. After a 3.5 h incubation (time 0 on the graph) a sample was removed for nuclear staining (Δ) and the remaining cells were transferred to medium containing anisomycin. At the time indicated by the line a sample of the culture was transferred to medium containing only hydroxyurea ($\bullet$) and another was transferred to fresh growth medium lacking inhibitors ($\circ$). Samples were removed for nuclear staining⁵.

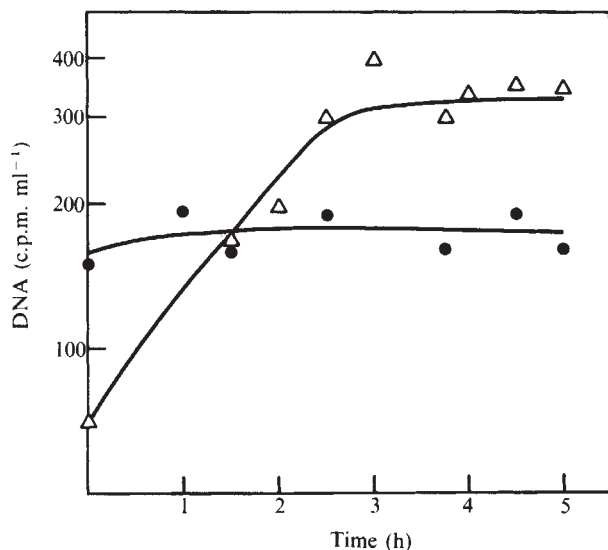


Fig. 3 Effect of anisomycin on the initiation of DNA synthesis in synchronized cells pretreated with hydroxyurea. A culture was synchronized by the starvation method of Williamson¹⁶ and inoculated at 5×10^5 cells ml⁻¹ into growth medium containing ¹⁴C-uracil (0.3 μ Ci ml⁻¹, specific 10 mCi mmol⁻¹). At 30 min before the second cycle of DNA synthesis (and budding) anisomycin was added to one portion of the culture and hydroxyurea to the other. After a 2 h incubation (time 0 on the graph) the cells incubated in the presence of hydroxyurea were transferred to medium containing anisomycin (Δ) while the other portion was incubated further with anisomycin ($\bullet$). Duplicate samples of 2 ml were analysed for radioactivity in DNA as previously described².

the absence of concomitant protein synthesis. This is consistent with and confirms the earlier results^{3,4}. Functional tests (mitosis or division) have detected failure to replicate the last 3% and 0.5% of the HeLa cell⁵ and *E. coli* genomes⁶, respectively. The nuclear division assay used here is analogous and demonstrates completion of DNA synthesis independently of the direct measures of DNA used by Williamson³.

The initiator proteins, presumably inactivated in the conditional mutant used by Hereford and Hartwell⁴, are shown here to be stable for 3.5 h in wild type cells. This is longer than the 3 h half life estimated for Chinese hamster cell initiator(s)⁷.

The ability to execute a complete round of DNA synthesis in the absence of concomitant protein synthesis is unusual for a eukaryotic cell⁸⁻¹⁰. Studies with the slime mould *Physarum polycephalum* indicate that the replication of 'early replicating DNA' is required for the synthesis of proteins which start the replication of other DNA¹⁰. Thus there is a causally connected ordered sequence of genome replication. This is obviously not true for DNA synthesis in yeast. A similar type of sequence may, however, exist for events in the 'DNA-division' cycle of yeast. The programmes leading to events which require DNA synthesis begin early in the cell cycle^{11,12}. The work with hydroxyurea shows that in its presence all the proteins needed for a full round of DNA synthesis are made. Some proteins, however, are not made for the events which depend on DNA synthesis (that is, cell division, new budding² and nuclear division). When hydroxyurea is removed, DNA is replicated and the proteins for the events which require DNA synthesis are made. This suggests, but does not prove, that the synthesis of proteins in the programme leading to DNA-dependent events are triggered by DNA synthesis which is triggered by the proteins made in the presence of hydroxyurea.

The unusual properties of DNA synthesis and nuclear composition (lack of lysine-rich histone from the nucleochromatin network^{13,14} and loose DNA packaging) might be clarified by studying the effects of inhibiting DNA synthesis on yeast 'histone' synthesis.

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Do D-Glucosamine and D-Galactosamine Form Part of Specific Receptor Sites for Competence Substance on Cell Wall of *Pneumococcus*?

D-GLUCOSAMINE and D-galactosamine probably form a part of the specific receptor sites for the competence substance on the cell wall of *Pneumococcus*, since these amino sugars completely inhibit the binding of the competence substance to the cell surface receptors, and, thus, the induction of competence in a genetically transformable pneumococcal strain.

The importance of the architecture of cell surfaces in determining biophysical interactions with other cells, with viruses, and with naked DNA molecules which may lead to gene flow from a donor to a recipient cell, has long been known, but information about the molecular mechanism of such interactions is still rather scarce. A specific surface structure (a different antigen) in each case differentiates male from female bacteria, cells which adsorb phage from those which do not, competent from non-competent populations of transformable bacteria, and malignant from normal cells. In all these cases the introduction of a new component seems to alter the surface charge of the cell bearing it, thus potentiating the various macromolecular interactions.

The significance of glycosyl residues seems to be that they confer a specific recognitional role on biopolymers. Antibody proteins of higher animals interact specifically with polysaccharides. A similar type of interaction has now been found with phytohaemagglutinins (PHA). PHA binds to carbohydrate structures on the cell surfaces and agglutinates erythrocytes and other types of cells, preferentially mammalian tissue culture cells that have been transformed by oncogenic viruses¹. These substances mimic antibody in that they interact to form a precipitate with biopolymers containing the 'correct' determinant sugars. Similarly, these sugars have been shown to inhibit the precipitation reaction. An interaction of concanavalin A (con A, a PHA of the jack bean) with the cell wall of *Bacillus subtilis* 168, containing glucosylated teichoic acid, has been found². In contrast, the cells of a *B. subtilis* strain which produces a teichoic acid devoid of glucose residues failed to interact with the PHA. A requirement for glucosylated teichoic acid for adsorption of phage in *B. subtilis* 168 has been reported³. Pretreatment of glucosylated cell walls with con A results in concentration-dependent inhibition of phage $\phi 25$ adsorption⁴.

We found that the agglutinins of *Pisum sativum* L. and *Lathyrus sativus* L. agglutinate transformable recipient cultures of *Diplococcus pneumoniae* and exhibit a concentration-dependent inhibition of the induction of competence by the competence substance in a young, not yet competent cell culture^{5,6}. This indicated that PHA and the competence might bind to the same receptor sites on the surface of pneumococcal cells. We have therefore tested a series of sugars to determine at least a part of the recognition site for the competence substance on the cell wall surface. We found that only D-glucosamine and D-galactosamine inhibit the induction of competence by the competence substance in a time-dependent manner. These same sugars do not interfere with subsequent stages of the transformation process. They therefore seem to be the determinants recognised by the competence substance on the surface of inducible pneumococcal cells.

The inhibition of induction of competence by D-glucosamine and D-galactosamine, as the function of the concentration of the amino sugars used, is seen in Fig. 1. In the presence of 5 mg of the respective amino sugars, 99.3% and 97% inhibition of induction of competence was recorded, as compared with the control without the sugar (with 5%

of transformants). The viability of the cells was not affected in the presence of the sugars.

A 97% and 99% inhibition of induction of competence was found when 5 mg of D-glucosamine and D-galactosamine, respectively, were first pre-incubated for 5 min with the saturating concentration of the competence substance, or when the respective sugars were added to the complex at zero time. In the case of the later additions, however, the inhibitory effect of both amino sugars decreased progressively with time, falling to zero at 21–22.5 min, when the induction of competence, in this particular case, was completed. These results (Fig. 2) demonstrate that the amino sugars interfere only at the time of the induction stage, that is the time of binding of the competence substance to the cell receptor sites. Further stages of the transformation process proceed in their presence without any disturbance.

A series of other sugars was tested, including D-glucose and D-galactose, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine, D-fructose, D-mannose D-arabinose and

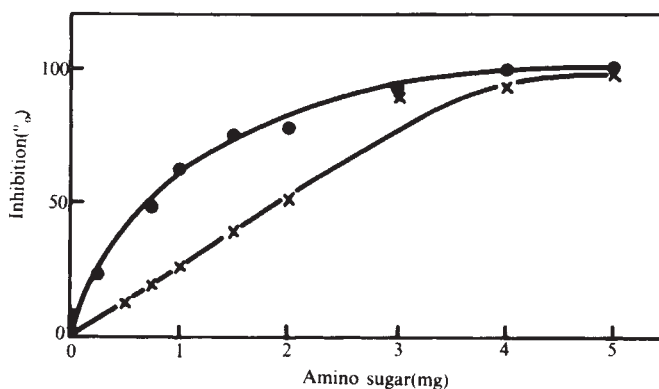


Fig. 1 The inhibition of induction of competence by the competence substance in the presence of amino sugars. D-Glucosamine HCl (●) and D-galactosamine HCl (x) were dissolved and diluted in 0.05 M phosphate buffer (pH 7) to obtain the required concentration, using 0.1 ml samples. Then 0.1 ml of the sample was preincubated for 5 min at 37° C in a water bath with 0.1 ml, or an aliquot amount of the competence substance at the saturating concentration. A sterile filtrate from a highly competent culture of *Diplococcus pneumoniae* of the R6-bd strain (a streptomycin-sensitive, sulphanilamide-resistant strain, isolated by Dr Tomasz, 40–60 min after the peak of competence has passed⁷, was used as the source of the highly active, competence-inducing substance. One millilitre of a physiologically young, yet incompetent culture of the above strain (transferred at A_{450} of 0.08–0.1 from the pretransformation into the transformation medium^{8,9} grown here for 20 min, corresponding to 3×10^6 viable units ml^{-1} , and chilled for 3 min (in ice) was then added and the cell culture suspension was induced for 20 min at 37° C in a water bath. In the control tube 0.1 ml of phosphate buffer was used instead of the solution of the tested sugar. In the control for incompetence of the recipient culture, used for induction of competence, 0.1 ml of the transformation medium was used, instead of the filtrate containing the competence substance. In a further control the filtrate with the competence substance at the saturating and subsaturating concentrations was used and the samples were completed to the given volume with the transformation medium. To all samples 0.05 ml of transforming DNA, bearing the streptomycin resistance marker (isolated from the streptomycin-resistant transformant of the R6-bd strain), at the plateau concentration level, was added and the tubes were incubated for 15 min at 37° C. The penetration of DNA was stopped by adding 0.05 ml of DNase (1 μg) in MgSO_4 (3 mM final concentration) to the suspension, which was incubated for further 3 min at 37° C. The tubes were then placed into ice, and after appropriate dilution of the samples, 0.1 ml aliquots were transferred on to plates with 0.2 ml sheep blood and mixed with a complete agar medium. After 2 h of incubation at 37° C the plates were overlaid with the agar medium containing 150 μg streptomycin sulphate ml^{-1} . Streptomycin-resistant transformants were scored after 16 h. 5% transformants were obtained in the control without the sugar (quoted always as percentage of added viables).

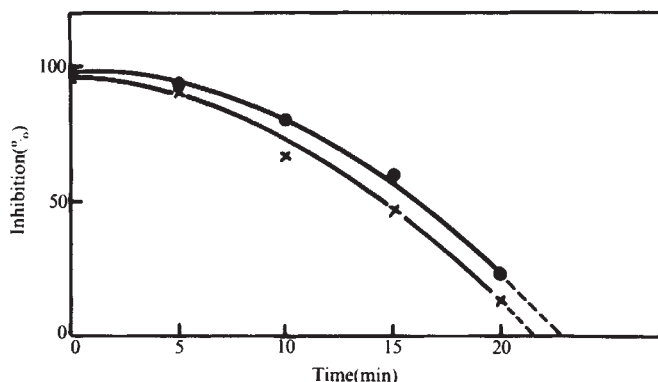


Fig. 2 The inhibition of induction of competence by the competence substance as the function of the time at which amino sugars were added to the competence substance-cell complex. 0.1 ml (5 mg) of the glucosamine (●) and of the galactosamine (×) were either preincubated for 5 min at 37° C with 0.1 ml of the saturating concentration of the competence substance, or the respective sugars were added at zero time, that is, immediately after the addition of the competence substance to the incompetent cells, and then to the 5, 10, 15 and 20 min competence substance-cell complexes, incubated at 37° C in the water bath. For induction 3×10^6 of incompetent cells (as viable units) were used. The frequency of transformants in the control was 5%.

L-fucose. Although all the sugars were tested under the same experimental conditions and at identical concentration levels, no inhibition but, rather, a stimulating effect on the induction and competence by the competence substance was found. Only with N-acetyl-D-galactosamine and with D-fructose was inhibition sometimes recorded (4–26% and 3–20% respectively), but in neither case was the degree of inhibition related to the concentration of the sugar.

The next question we posed was whether the amino sugars inhibit the induction of competence because they are the natural receptors (or determinant groups) of the competence substance to which they bind, or, alternatively, because their free amino groups compete with the competence substance for identical receptor sites at the cell surface. It has been reported that a polyamine might be an integral part of the highly basic polypeptide molecule of the competence substance¹⁰.

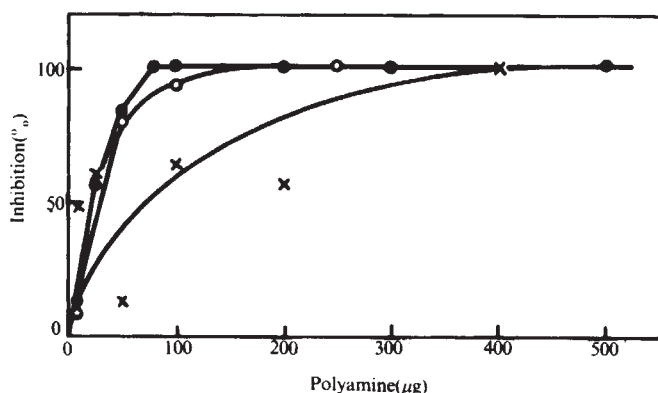


Fig. 3 The inhibition of induction of competence in the presence of protamine sulphate (●), spermine tetra-HCl (○) and histone sulphate (×). The respective substances were dissolved in the phosphate buffer and treated as described in the legend to Fig. 1. 1.3×10^6 incompetent cells were used for the induction. The frequency of transformants in the control was 1%. In the presence of 0.5 mg of protamine only 20% and in the presence of 1 mg of histone only 50% of cells (as viable units), as compared with the control without the polyamine, was found.

We investigated, therefore, whether basic substances of polyamine nature, such as protamine and spermine and the basic protein histone, would interfere with the induction process. Using the same experimental conditions as above we found that protamine and spermine completely inhibited the induction of competence, even at concentration levels of 0.08, 0.2 and 0.4 mg respectively, as can be seen in Fig. 3. But, unlike the amino sugars, they interfered not only with the induction stage, but also with further stages of the transformation process. Thus 0.1 mg of spermine and protamine produced 73% and 98% inhibition of transformation respectively when added to an already competent cell culture. All three basic substances strongly agglutinated incompetent cells, in parallel with their inhibiting effect on the induction of competence, and also produced a marked reduction in the viable cell count. It seems that these basic substances interact with the competence substance receptor sites on the cell surface, and may thus be responsible for some non-specific conformational changes which result in agglutination of the cells and a concomitant decline in the cell count.

The effect of choline, a constituent of the teichoic acid of the pneumococcal cell wall^{11–13} and of its analogue, ethanolamine¹⁴, on induction of competence was also investigated. A stimulatory effect of choline (Fig. 4a) and an inhibitory effect of ethanolamine (Fig. 4b) were recorded. When 5 mg of the ethanolamine were added to fully competent cells a 30% inhibition in the yield of transformants was found. The viable count decreased in the presence of 2 mg choline to 50%, and in the presence of ethanolamine to 33%, as compared with the viable count in the sample with the competence substance alone.

Two further experiments were performed to determine whether the amino sugars inhibit the induction of competence by binding directly to the competence substance. In the first, 1 mg and 5 mg galactosamine were pre-incubated with varying concentrations of competence substance ranging from subsaturating (0.05 ml) to hypersaturating (0.15–0.3 ml). The inhibitory effect of the amino sugar was progressively reduced as the concentration of competence substance rose.

Although this experiment suggests a direct interaction between amino sugar and competence substance, it by no means excludes a competition between them for receptor sites. A second, more direct experiment was therefore devised. The proposition put forward was as follows: if the amino sugars and the competence substance bind to identical surface receptors, these should be blocked in the presence of 5 mg D-galactosamine so that, after centrifuging, the sedimented cells should not be inducible by newly added competence substance. On the other hand, if the amino sugars bind to the competence substance, the receptors should be unoccupied so that, upon centrifugation, the amino sugar-competence substance complex should remain in the supernatant. The sedimented incompetent cells, their receptors unoccupied, should thus be inducible by newly added competence substance.

The results of the experiment were clear cut and showed that the sedimented cells, after 5 min pretreatment with D-galactosamine and saturating concentration of the competence substance could be induced by newly added competence substance to the same degree as the control without added sugar. In contrast, 100% inhibition of induction was observed in the control containing 5 mg D-galactosamine throughout the whole procedure. We infer that the inhibitory effect of the amino sugar results from its binding to, and inactivation of, the competence substance, and not to an affinity for cell receptors.

The results presented here indicate that D-glucosamine and D-galactosamine, in their non-acetylated form, are an integral part of the cell surface receptors and seem therefore to be the determinants, recognised by the competence substance, on the surface of inducible pneumococcal cells. One of the

still unresolved questions to be answered is whether the free amino groups of the sugar receptors are consistent with the physiological state at which the competence substance binds to them, or whether a deacetylase has first to remove the acetyl groups from the amino groups of D-glucosamine and D-galactosamine. Only N-acetylated galactosamine and glucosamine have so far been reported to form part of the C-polysaccharide or teichoic acid of the peptidoglycan 'backbone' in pneumococcal cell wall^{12,13,15} and of the teichoic acid of the cell wall in highly transformable strains of *Bacillus subtilis*¹⁶. On the other hand, N-non-substituted glucosamine residues in *Bacillus cereus* cell wall peptidoglycan were demonstrated and accounted for resistance of the cell walls to lysozyme¹⁷.

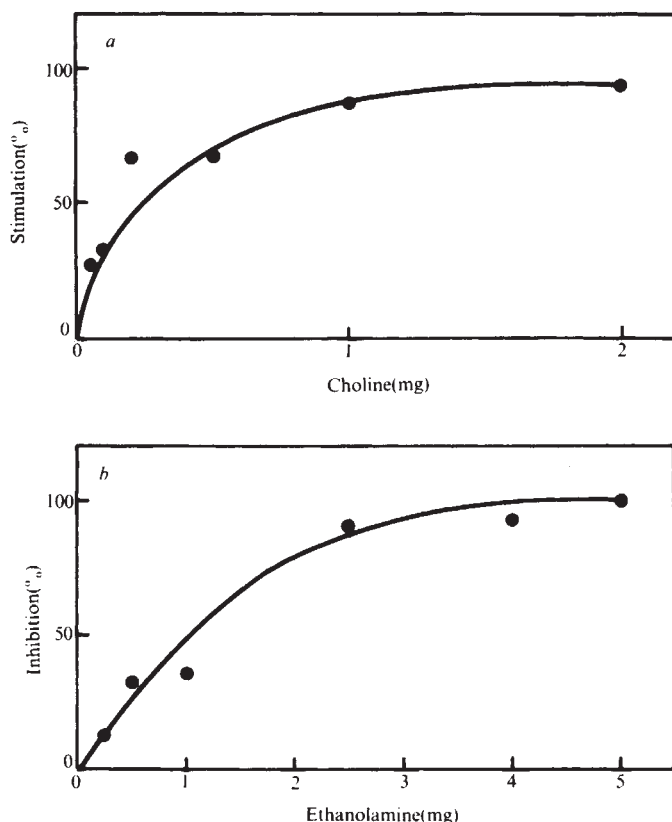


Fig. 4 The effect of choline (a) and of ethanolamine (b) on the induction of competence by the competence substance. Choline was dissolved and diluted in the phosphate buffer (pH 7) to obtain the required concentrations, using always 0.1 ml samples. Ethanolamine was diluted in the same buffer and the pH was adjusted with concentrated HCl to 7. Procedure was as in the legend to Fig. 1. 1.3×10^6 viable units ml^{-1} of the incompetent cell culture was used. The frequency of transformants in the control was 1%.

The presence of a 2-N-acetamido-4-amino-2,4,6-trideoxyhexose was also reported in the competence substance¹⁸. The sterical arrangement of the adjacent $-\text{NH}_2$ and $-\text{OH}$ groups in this sugar might be identical with that of $-\text{NH}_2$ and $-\text{OH}$ groups both in D-glucosamine and D-galactosamine. Therefore this diamino sugar can also not be excluded as a potential determinant. Investigations of this point are on the way.

The positively charged competence substance bound to its amino sugar determinants on the cell wall surface would not only impose a change in the surface charge from negative to positive, and thus enable the reversible adsorption of the negatively charged DNA, but possibly also, by introducing some conformational changes into the stereochemistry of the cell wall polymers, trigger off the autolytic enzyme

activity. Thus, weak sites for the penetration of DNA to the deeper cell structures might be produced.

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Dissociation of Cell Division Stimulating Capacity for Balb/c-3T3 from the Insulin-like Activity in Human Serum

TEMIN *et al.*^{1,2} have shown that insulin and serum proteins with insulin-like activity, isolated from either human or calf serum stimulate cell division in diploid populations of chick cells, while virus-transformed cells grow in the absence of these factors. An established line of rat liver cells, with a unique ability to grow in the absence of serum, releases macromolecular factors into the medium that have insulin-like activity and induce non-transformed cells to divide^{3,4}; this suggests again that the factors with insulin-like activity are required to initiate cell division. Crystalline insulin stimulates DNA synthesis in BHK cells at limiting serum concentrations⁵, and is a requirement for growth in Agarose of some non-transformed variants of these cells⁶. Both insulin and whole serum have a pleiotropic effect on non-transformed 3T3 cells⁷; they induce RNA and protein synthesis, and prevent protein degradation. These factors are not needed for the maintenance of the pleiotropic effect in virus-transformed cells⁷. In addition, insulin and serum⁸ both cause a rapid decrease in the concentration of intracellular cyclic AMP in 3T3 cells, the decline of which may be a prerequisite for cell division^{9,10}. We have isolated a serum fraction containing the factors that stimulate DNA synthesis and have insulin-like activity, and have found that heating separates the two activities.

Clonal Balb/c-3T3 cells were maintained on Falcon microtitre wells (area 0.3 cm^2) in Dulbecco's modification of Eagle's medium containing 4.5 g of glucose per litre and 2% calf serum (Colorado Serum Company). To study DNA synthesis, ³H-thymidine and protein human serum fractions

were added to these cultures 5–7 d after the medium was changed. Forty-eight hours later, the cells were fixed and processed for autoradiography; cell division was determined in the same cultures by determining the total cell number with the aid of a microscopic grid¹¹. The incorporation of ^{14}C -U-glucose into glycogen by the muscles of the rat diaphragm *in vivo* was used as an index of insulin-like activity as described by Rafaelsen *et al.*¹². To isolate the active fraction, whole blood was collected from healthy humans, allowed to clot and the serum was separated by centrifugation. The factors that induce DNA synthesis and exhibit insulin-like activity were extracted from the serum by ion exchange chromatography using a Dowex-50w-x8 ion exchange resin (Na^+ cycle) as described by Antoniadou¹³. The adsorbed fraction was eluted with 0.1 M NaHCO_3 buffer (pH 9.5), neutralised with 0.36 N HCl and concentrated using Amicon Diaflow membranes (UM-10); the concentrate was dialysed against 0.15 N NaCl and lyophilised. Approximately 8 mg of protein were routinely obtained from 100 ml of human serum.

Figure 1 shows that crystalline insulin and the Dowex-adsorbed serum protein fraction both stimulated the incor-

poration of glucose into glycogen *in vivo*, by the rat diaphragm. Crystalline insulin was not responsible for the effect of the serum fraction, since none was detectable in these preparations by competitive radioimmunoassay¹⁴. Heating the serum fraction to 100°C for 20 min destroyed the factor(s) with insulin-like activity as determined by the incorporation of glucose into glycogen (Fig. 1). In contrast, the factor(s) that stimulate DNA synthesis in confluent cultures of Balb/c-3T3 were not destroyed during 20 min at 100°C (Fig. 2). Approximately 90 μg of protein stimulated DNA synthesis in 50% of the cells representing a ten-fold purification compared with whole serum. The factor(s) that stimulate cell division were also resistant to 100°C since the total cell number present 48 h after the addition of 200 μg of either heated or untreated serum protein was 55% greater than the control. Crystalline insulin ($3\text{--}100\text{ mU ml}^{-1}$) did not stimulate DNA synthesis in these confluent cultures, nor did proinsulin, protamine insulin or bovine growth hormone (Armour).

The Dowex-adsorbed serum fraction could not be used as a complete serum substitute, since freshly planted Balb/c-3T3 cells did not adhere to the plate and did not survive for 24 h, even in the presence of 5% (w/v) human serum albumin. In contrast, most of the serum proteins, that did not bind to the ion exchange resin, supported the growth of sparse populations of Balb/c-3T3, but the final saturation density of these cells was only 50% of those in an equivalent concentration of whole serum. This was not entirely unexpected since Holley¹⁵ and others^{16,17} have demon-

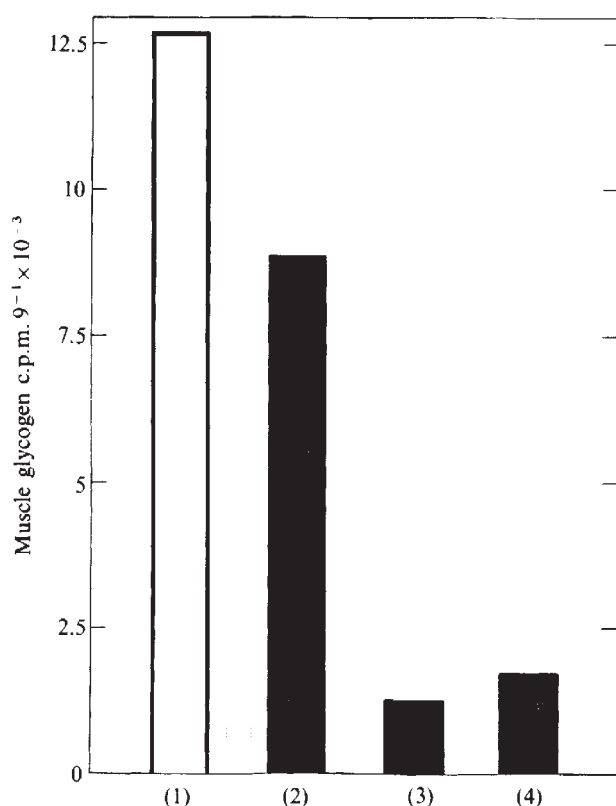


Fig. 1 The effect of heating on the serum insulin-like activity. The Dowex-50w-x8 adsorbed serum fraction was dissolved in 0.15 M NaCl (concentration 2 mg ml^{-1}) and a portion was kept at 100°C for 20 min. Insulin activity was determined by the synthesis of glycogen by the rat diaphragm using crystalline porcine insulin as a standard^{12,14}. The heated or untreated serum fraction (1.6 mg of protein) or insulin was dissolved in Gey's bicarbonate buffer containing 5% (w/v) human serum albumin and together with 2 μCi of ^{14}C -U-glucose (3.3 mCi mmol^{-1}) was injected intraperitoneally (volume, 5 ml) into a non-fasted Sprague-Dawley rat (120–130 g); the buffer control did not contain the added protein fraction. Two hours later the animal was killed, its diaphragm removed, weighed, hydrolysed in 30% (w/v) KOH at 100°C , and the supernatant clarified by low speed centrifugation. The glycogen was precipitated by the addition of Na_2SO_4 to 12 mM and absolute ethanol to a final concentration of 70% (v/v); the c.p.m. represent the mean of five replicate determinations. Column (1) insulin, 1,000 μU ($40\text{ }\mu\text{g}$); column (2) adsorbed protein; column (3) adsorbed protein kept at 100°C for 20 min; column (4) buffer control.

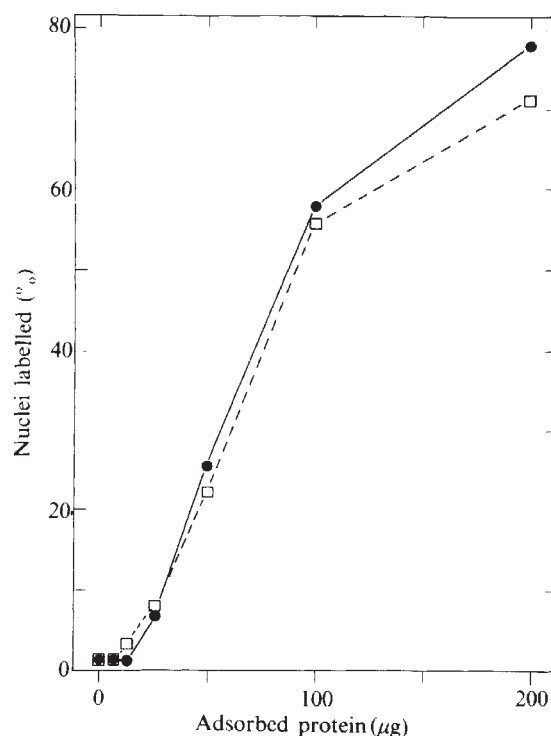


Fig. 2 The effect of serum on the DNA synthesising capacity. The Dowex-50w-x8 adsorbed serum fraction, which was heated at 100°C for 20 min as described in Fig. 1 (□) or left untreated (●), was added to confluent cultures of an early passage of Balb/c-3T3 (clone A31) in cell depleted²² medium (total volume, 0.2 ml) containing 2% (v/v) calf serum, and ^3H -TdR $5\text{ }\mu\text{Ci ml}^{-1}$ (6.7 Ci mmol^{-1}). Forty-eight hours later, the cells were fixed with a mixture of cold ethanol and glacial acetic acid (3:1 v/v), washed three times with 5% trichloroacetic acid at 4°C , once with methanol and air-dried. The microtitre wells were punched out and glued to a glass slide with the cell side up. The cells were overlaid with NTB2 photographic emulsion (Kodak), dried over CaCl_2 and allowed to set for 2 d; after fixation they were counterstained with haematoxylin. Two hundred cells were counted for each determination, while $\sim 10^4$ were scanned.

strated that separate serum factors are needed for the growth, survival and movement of nontransformed 3T3 cells.

These experiments demonstrate that the serum factor(s) that stimulate DNA synthesis and cell division in confluent cultures of Balb/c-2T3 are stable to 100° C and are therefore distinct from the factor(s) with insulin-like activity which are destroyed at this temperature. Pierson and Temin² have used cation exchange chromatography to isolate factor(s) from calf serum that stimulate cell division in nontransformed populations of cells; these proteins are sensitive to sulphhydryl reduction and proteolytic degradation, and are also stable to 80° C and acid. A factor from human serum that stimulates cell division has recently been separated from the heterogeneous group of factors with insulin-like activity by the use of preparative isoelectric focusing (our manuscript in preparation), demonstrating again that they are distinct molecules.

Pasten *et al.*⁹ and Burger *et al.*¹⁰ suggested that a decrease in the concentration of intracellular cyclic AMP in cultures of fibroblasts is an initiating event in cell division. A decrease in intracellular cyclic AMP, however, is not itself sufficient to stimulate cell division since crystalline insulin (80 mU ml⁻¹) rapidly lowers intracellular cyclic AMP in confluent cultures of Balb/c-3T3⁸, without inducing subsequent DNA synthesis. Whole serum decreases intracellular cyclic AMP concentrations and induces subsequent cell division but contains insulin and proteins with insulin-like activity. Partially purified human serum preparations with insulin-like activity (termed somatomedin¹⁸) induce DNA and chondroitin sulphate synthesis in isolated costal cartilage from hypophysectomised rats. These fractions compete with insulin for membrane binding sites on isolated liver, fat and cartilage cells¹⁹ and, like insulin, have been shown to inhibit the basal activity of adenyl cyclase in cellular membrane preparations^{20,21}. Since heating destroys the factors with insulin-like activity present in human serum without affecting the factors that stimulate cell division, it may be possible for us to help clarify the role of cyclic AMP in the cellular growth cycle by learning if the serum factors that induce DNA synthesis also decrease the intracellular concentration of cyclic AMP.

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p-Fluorophenylalanine and 'Division-related Proteins'

CELLS approaching mitosis after completion of DNA synthesis are believed to synthesise certain RNA messages and to translate these in order that proteins essential for mitosis become available¹⁻⁶. The synthesis during G₂ of 'division-related proteins' is by no means proven, however, and there is distinctly contradictory evidence in the case of HeLa cells⁷. The time spent in G₂ varies considerably between cell types and the synthesis of proteins involved in the mitotic process might be sufficiently complete by mid S phase in some cell lines while it is only accomplished by late G₂ in others. If we assume, however, that proteins specifically related to division are made in G₂, then it is of interest to know the latest time that they can be made before division, and what they do.

Some studies of the G₂ phase have involved the amino acid analogue *p*-fluorophenylalanine^{2,8,9}. Experiments with bacteria¹⁰ suggest this analogue could resolve some of the problems of division proteins, but similar experiments have not been carried out in eukaryotic cells. We analysed the effects of *p*-fluorophenylalanine on the progression of HeLa cells from G₂ into mitosis, restricting our interest to the requirements of cells to reach metaphase.

When cells incorporate an analogue such as *p*-fluorophenylalanine, the altered proteins may malfunction^{10,11}. If proteins made in G₂ are essential for sending cells into division, those synthesised in the presence of *p*-fluorophenylalanine might prevent cells entering mitosis. We have carried out this experiment many times with *p*-fluorophenylalanine in HeLa cells. To avoid competition between phenylalanine and *p*-fluorophenylalanine, a precaution not adopted by others^{2,8,9}, cells were initially transferred to a phenylalanine-free Eagle's HeLa medium containing 10% dialysed calf serum 2 h before experimentation. This deprivation produced a slowly increasing inhibition of cell cycling only after 6-7 h. When DL-*p*-fluorophenylalanine was added at a concentration equivalent to that of phenylalanine in basal Eagle's medium (0.2 mM, 0.1 mM with respect to the L-form), entry of cells into mitosis was completely inhibited within about 15 min (Fig. 1a) and prophase rapidly disappeared from the cultures. Lower concentrations were less inhibitory, 0.05 mM being relatively ineffective. When competition between DL-*p*-fluorophenylala-

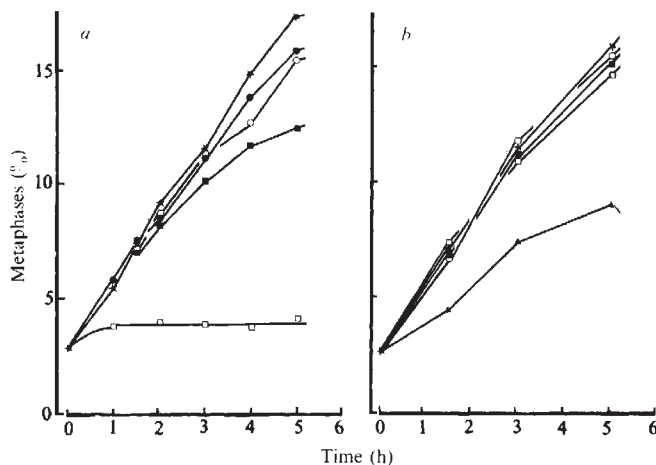


Fig. 1 *a*, Effect of *p*-fluorophenylalanine in phenylalanine-free medium on entry of cells into mitosis. Suspension cultures of HeLa S-3 cells were transferred to phenylalanine-free medium 2 h beforehand and at time 0 h, colcemid (1.5×10^{-7} M) was added. Mitotic activity is expressed as percentage metaphases from duplicate counts of 1,000 cells at each time point. Symbols, which are common to all the figures, are: ★, Control, containing 0.1 mM L-, or 0.2 mM DL-, phenylalanine; ●, phenylalanine-free medium; ○, 0.05 mM *p*-fluorophenylalanine in phenylalanine-free medium (phenylalanine medium for Figs 1*b* and 2*b*); ■, 0.1 mM *p*-fluorophenylalanine in phenylalanine-free medium (phenylalanine medium for Figs 1*b* and 2*b*); □, 0.2 mM *p*-fluorophenylalanine in phenylalanine-free medium (phenylalanine medium for Figs 1*b* and 2*b*); ▲, 0.5 mM *p*-fluorophenylalanine in phenylalanine-free medium (phenylalanine medium for Figs 1*b* and 2*b*). Where dashed lines join the points, the same symbols apply but the cultures were incubated at $40 \pm 0.2^\circ$ C, not $37 \pm 0.2^\circ$ C. The graph for 0.5 mM *p*-fluorophenylalanine is omitted as it falls away to the abscissa in 1–2 h. *b*, Relatively ineffective action of *p*-fluorophenylalanine at the same dose levels described in Fig. 1*a* when added in competition with 0.2 mM DL-phenylalanine in the medium.

nine and DL-phenylalanine was allowed, a 2.5 to 5.0-fold excess of the analogue was required to obtain an inhibitory effect (Fig. 1*b*).

There is evidence that *p*-fluorophenylalanine is incorporated into bacterial proteins^{12,13} and that there is some suppression of protein synthesis by the analogue¹⁴. It has been surmised that the analogue is similarly incorporated into proteins of mammalian cells^{2,8,9} although this has only been shown in rabbit haemoglobin¹⁵, and serum, liver and muscle proteins¹⁶. Our results (to be published) show that incorporation also occurs into HeLa cell proteins. Thus the results of the above experiment, which agree with previous findings⁹, suggest that proteins made during G_2 are rendered faulty, or their synthesis is inhibited, by *p*-fluorophenylalanine, thereby preventing entry of cells into division.

Smith and Pardee¹⁰ found that bacteria incorporating *p*-fluorophenylalanine have an increased thermolability. Since *p*-fluorophenylalanine enters the proteins of HeLa cells, a similar thermolability might develop. Cultures were grown in the presence of DL-*p*-fluorophenylalanine at several concentrations up to the fully inhibitory level of 0.2 mM. One of each pair of cultures was kept at 37° C, the other being raised to 40° C in a Gyrotory shaker (New Brunswick Scientific Co.). Figure 2*a* shows the enhancing effect of increased temperature on the inhibitory action of low levels of *p*-fluorophenylalanine. This experiment, like the first, leads to the conclusion that 'division-related proteins' are synthesised in G_2 . *p*-Fluorophenylalanine incorporated in competition with phenylalanine which were inadequate to stop entry into mitosis at 37° C also proved inhibitory at the elevated temperature (Fig. 2*b*).

Preliminary studies with inhibitors showed that at $5 \mu\text{g ml}^{-1}$, cycloheximide and puromycin suppressed protein synthesis by

Table 1 Amino Acid and Analogue Incorporation into HeLa Cell Protein in the Presence and Absence of Cycloheximide and Puromycin.

Time of sample	Cycloheximide ($5 \mu\text{g ml}^{-1}$)/ puromycin ($2 \mu\text{g ml}^{-1}$)	Specific activity as c.p.m. per μg protein (% of control)		
		^3H -Leu	^{14}C -Phe	^{14}C - <i>p</i> -Phe
2	—	38	77	81
4	—	56	171	130
2	+	5 (13%)	9 (12%)	8 (10%)
4	+	5 (9%)	13 (8%)	11 (8%)

Suspension cultures of HeLa S-3 cells were pulsed with $4,5\text{-}^3\text{H}$ -L-leucine (Radiochemical Centre, Amersham, specific activity 17 Ci mmol^{-1}) for 30 min before the time point at $1.0 \mu\text{Ci ml}^{-1}$. Cells were washed twice with ice-cold saline, treated with 4% perchloric acid and the insoluble residue washed with 80% ethanol, 100% ethanol, and ethanol/ether. After drying, the residue was taken up in 90% formic acid and aliquots taken for protein estimation and radioactive counting in a xylol/dioxane/ethanol based scintillation mixture. A Packard 2425 spectrometer was used.

$1\text{-}^{14}\text{C}$ -DL-3-phenylalanine (Radiochemical Centre, Amersham, specific activity 59 mCi mmol^{-1}) was added at $0.04 \mu\text{Ci ml}^{-1}$ to HeLa suspension cell cultures which had been prestarved of phenylalanine for 2 h. The labelled amino acid was diluted with cold DL-phenylalanine to give a final concentration of 0.2 mM. This was continuously present and samples were removed at 2 h and 4 h, and treated as described above.

$3\text{-}^{14}\text{C}$ -DL-*p*-fluorophenylalanine-HCl (Calatonic, Los Angeles, California, specific activity 16 mCi mmol^{-1}) was given in an identical manner to ^{14}C -phenylalanine at $0.04 \mu\text{Ci ml}^{-1}$ after dilution with cold DL-*p*-fluorophenylalanine to a final concentration of 0.2 mM.

80–90% without causing significant cell death over 4–5 h. The best results were achieved, however, with a combination of $5 \mu\text{g ml}^{-1}$ cycloheximide + $2 \mu\text{g ml}^{-1}$ puromycin, which suppressed protein synthesis by more than 90% as measured by pulses of ^3H -leucine at 4 h, without producing significant cell death (Table 1). Incorporation of ^{14}C -*p*-fluorophenylalanine was inhibited after 4 h by 90% in exactly the same manner as ^{14}C -phenylalanine and ^3H -leucine (Table 1). Experiments similar to those described earlier were then repeated with cultures receiving cycloheximide/puromycin treatment. The results show conclusively that inhibition of *p*-fluorophenylalanine incorporation prevents the analogue from (1) exerting its inhibitory effect on the entry of cells into mitosis (Fig. 3*a*) and (2) increasing the thermolability of proteins (Fig. 3*b*).

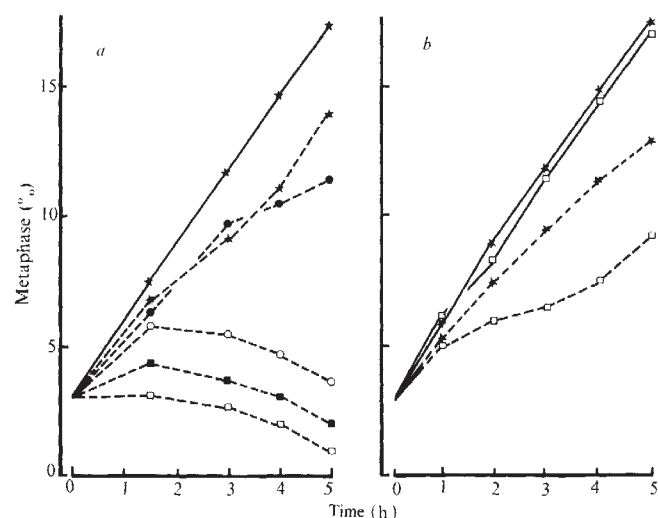


Fig. 2 *a*, Influence of raised temperature ($40 \pm 0.2^\circ$ C) on entry into mitosis of HeLa S-3 cells. Details as for Fig. 1*a*. *b*, As for (a) except, as in Fig. 1*b*, competition was allowed between 0.2 mM DL-phenylalanine and 0.2 mM DL-*p*-fluorophenylalanine.

Further inferences can be drawn from these experiments. First, the physical presence of *p*-fluorophenylalanine within the cell in the absence of protein synthesis does not prevent cell progression into mitosis, that is, the inhibitory action of *p*-fluorophenylalanine is only manifest after it has become incorporated into cellular proteins. Second, the experiments verify the observation repeatedly found in this laboratory and elsewhere⁷ that inhibition of protein synthesis during G₂ does not prevent HeLa cells from reaching metaphase (Fig. 3a). Although cycloheximide/puromycin treatment along with raised temperature produced an accelerated loss of metaphases as pyknotic cells after 3 h, which accounts for the rapid fall-off in the lower curves of Fig. 3b, prophase were still appearing in these cultures at about 80% of the control rate by 5 h.

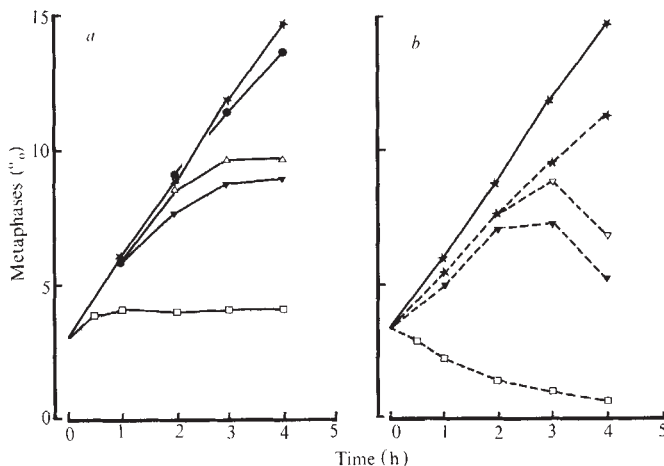


Fig. 3 a, Effect of inhibition of protein synthesis on the action of 0.2 mM *p*-fluorophenylalanine in phenylalanine-free medium. Symbols as for Fig. 1 except (△) which is cycloheximide 5 µg ml⁻¹ + puromycin, 2 µg ml⁻¹ added to phenylalanine-free medium, and (▼), cycloheximide/puromycin added to phenylalanine-free medium containing 0.2 mM *p*-fluorophenylalanine. b, As for Fig. 3a but cultures incubated at 40°C where dashed lines have been used. ▽ --- ▽, Cycloheximide/puromycin treatment of control culture, in this case containing phenylalanine.

The relationship of *p*-fluorophenylalanine to the hypothetical 'division-related' proteins needs re-examination. We tentatively offer the following explanation of our results. HeLa cells entering G₂ already have sufficient amounts of the proteins required for entry into, and completion of (unpublished data), a mitotic division, as evidenced by the inability of non-lethal doses of cycloheximide and puromycin to arrest the progression of G₂ cells into mitosis (Fig. 3a and ref. 7). When cells are exposed to *p*-fluorophenylalanine, they synthesise anomalous proteins. The presence of a small, but critical amount of these anomalous proteins in the pool leads to malfunctioning of the proteins in this system as a whole and cells fail to reach mitosis. The critical level for interference by *p*-fluorophenylalanine-containing proteins is lowered when cells are incubated at a higher temperature because an even greater instability is imparted to the whole system under these conditions. When the synthesis of anomalous proteins is inhibited, G₂ cells progress normally into mitosis. There is no evidence that *p*-fluorophenylalanine *per se* prevents cells reaching division.

HeLa cells may still be preferentially incorporating *p*-fluorophenylalanine during G₂ into proteins involved in division although this synthesis has been shown to be superfluous to the needs of the cell for entering mitosis. We are examining the proteins synthesised in G₂ in *p*-fluorophenylalanine-containing medium, and the incorporation of ¹⁴C-*p*-fluorophenylalanine into G₂ proteins. In other cell lines where the transition point from sensitivity to resistance to protein synthetic

inhibitors lies closer to mitosis, the effect of *p*-fluorophenylalanine incorporation may have quite different consequences from those seen in HeLa cells. For this reason we are presently studying its effects on CHO cells³.

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Reduction in Brain Dopamine following Experimental Cerebral Ischaemia

ALTHOUGH considerable information is available concerning the effects of discrete electrolytic lesions on the concentrations of the monoamine neurotransmitters in mammalian brain, little is known about the neurochemical effects of experimental vascular lesions. Such information would be useful in understanding the natural history of cerebral infarction and other neurovascular diseases; furthermore, it might suggest new therapies for treating the acute and chronic sequelae of such diseases. The observations reported here show that ligation of the middle cerebral artery in monkeys, or of the common carotid artery in gerbils, causes profound decreases in brain dopamine but not nor-adrenaline.

Six squirrel monkeys (*Saimuri sciura*) were subjected to transorbital ligation of the left middle cerebral artery, as described by Hodgins *et al.*¹. Three hours later they were guillotined and the left and right cerebral hemispheres assayed for dopamine by the method of Carlsson and Waldeck². Ipsilateral to the vascular lesion, the brain dopamine concentration was 0.64 ± 0.10 µg g⁻¹; in the contralateral hemisphere, this concentration was 1.13 ± 0.15 µg g⁻¹ ($P < 0.02$).

In developing an experimental preparation that might allow us to accumulate data from relatively large numbers of animals, we examined brain catecholamines in the Mongolian gerbil (*Meriones unguiculatus*). The blood supply to the gerbil brain is unique in lacking connecting arteries between the basilar and carotid circulations; hence, unilateral hemispheric infarction can be produced by ligating a common carotid artery³. A 50% mortality rate, equal to the infarction rate, is observed within 5 d of ligation⁴. Before death, animals may exhibit various clinical signs of infarction, in-

cluding external rotation of paretic limbs when walking, hypokinesia of the contralateral side of the body, ptosis of the contralateral eyelid, and circulating behaviour, usually towards the ischaemic hemisphere and around a progressively decreasing diameter⁴.

Mature mongolian gerbils (60–70 g) were anaesthetised lightly with diethylether, and the left common carotid was exposed through a ventral midline cervical incision. After the vagus nerve and the jugular vein had been dissected free, the artery was doubly ligatured with 4–0 silk sutures, and transected as previously described³. Groups of twelve animals were guillotined after various intervals, and their left and right cerebral hemispheres assayed for dopamine² and noradrenaline⁵. Brains from other animals prepared similarly were dissected into neostriatum, nucleus accumbens plus olfactory tubercle, hypothalamus, and the rest of the hemisphere, and pooled samples ($n=4-5$) were assayed for dopamine and noradrenaline. Twenty-four hours after unilateral carotid ligature, brain dopamine was reduced by 46% on the infarcted side ($2.09 \pm 0.39 \mu\text{g g}^{-1}$ compared with $0.97 \pm 0.24 \mu\text{g g}^{-1}$, $P < 0.02$). Brain dopamine was not significantly reduced among gerbils killed 2 h after lesioning, but was depressed among animals killed after 3 h. Brain noradrenaline concentrations were not dissimilar ipsilateral and contralateral to the lesion (that is, $1.19 \pm 0.076 \mu\text{g g}^{-1}$ compared with $1.04 \pm 0.093 \mu\text{g g}^{-1}$) among animals killed after 24 h.

Table 1 Effects of Ligaturing Left Common Carotid Artery on Regional Brain Dopamine Concentrations in Gerbils

	Sham-operated $\mu\text{g g}^{-1}$		Common carotid ligature $\mu\text{g g}^{-1}$	
	Left	Right	Left	Right
Neostriatum	1.12, 1.15	1.19, 1.18	0.69, 0.83	1.05, 1.06
Hypothalamus	1.86, 1.93	1.75, 1.83	0.80, 0.70	1.75, 1.19
Nucleus accumbens- olfactory tubercle	1.30, 1.59	1.40, 1.29	0.91, 0.71	1.58, 1.32
Rest of hemisphere	0.35, 0.37	0.34, 0.31	0.24, 0.14	0.13, 0.37

Two experiments were performed using groups of five and four animals respectively; tissues were pooled prior to assay. Animals were killed 24 h after placement of lesions.

Large brain dopamine reductions were observed in three parts of the cerebrum known to receive dopaminergic projections⁶, but apparently not in the rest of the hemisphere (Table 1). The largest decreases were in the hypothalamus and the nucleus accumbens-olfactory tubercle (Table 1). Among sham-operated animals in which the common carotid artery was exposed, but not ligatured, brain dopamine was unchanged in all regions examined (Table 1).

Subtotal ischaemic necrosis of the telencephalon and diencephalon ipsilateral to the vascular lesion probably accounts for the profound decreases in dopamine concentrations within these brain regions. The area of infarction would be expected to include several dopaminergic tracts, that is, the nigro-neostriatal pathway, the tubero-infundibular pathway, and the cephalic portion of the meso-limbic pathways^{3,4}. The major difference in the apparent susceptibility of noradrenergic and dopaminergic neurones to the ischaemia produced by left common carotid ligature could result from any of the following mechanisms: (1) the caudal location in the brain stem of noradrenergic cell bodies, which would continue to be nourished by the basilar artery; (2) the existence in gerbils of a single anterior cerebral artery, which would allow noradrenergic terminals in the anterior pole of the left hemisphere to be nourished by blood from the right carotid artery, and (3) differences in the susceptibility of noradrenergic and dopaminergic neurones to anoxia. The blood supply to the gerbil hypothalamus is provided by a rather large and consistent end artery derived from the anterior circulation; it is not surprising that the decrease in dopamine is most marked in this region.

Dopamine liberated from infarcted or severely ischaemic neurones could diffuse beyond synaptic clefts and interact with intracerebral blood vessels, glia, or as a false neurotransmitter with neurones that do not normally receive dopaminergic inputs. Since the inactivation of dopamine by oxidative deamination and by re-uptake into presynaptic neurones both require oxygen, the physiological effects of any dopamine released within ischaemic areas might be expected to be potentiated. Osterholm and his colleagues have suggested that, following experimental trauma to the spinal cord, noradrenaline liberated within the injured tissue causes intraspinal blood vessels to constrict, and thereby exacerbates tissue damage⁷. While the effects of dopamine on cerebral blood vessels await characterisation, this amine is known to modify vascular tone in peripheral organs⁸; hence, the possibility that brain blood flow is altered by the release of very large amounts of the amine after strokes merits exploration.

Just as the excessive release of dopamine might contribute to the acute pathophysiology of middle cerebral artery strokes, the neurological status of animals and humans who survive such strokes might also be complicated by a chronic decrease in central dopaminergic tone. That changes in brain dopamine similar to those reported here may also occur in human patients is suggested by the observation that the internal capsule of the human brain contains high concentrations of homovanillic acid⁹, and thus probably dopaminergic neurones.

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Glucose Inhibition of the Glucose-sensitive Neurone in the Rat Lateral Hypothalamus

It is generally accepted that the lateral hypothalamic area (LH) and ventromedial nucleus (VMH) have a reciprocal relationship in the control of feeding behaviour¹⁻³, and that some neurones in these regions are sensitive to levels of glucose in the blood^{4,5} or to electro-osmotically applied glucose⁶. We have demonstrated that although about one-third of the VMH neurones increased their activity when glucose was

Table 1 Number of Neurones in which Glucose-induced Inhibition was Blocked by Ouabain or Sodium Azide

	No. of neurones tested	Blocked	Unblocked
Ouabain	7	4	3*
Sodium azide	5	4	1
	12	8	4

* Application was impossible in all three because the resistance of the ouabain pipette was more than 10^3 M Ω and this was beyond the compensation capacity of the constant current device with a maximum output voltage of ± 100 V (ref. 12).

applied, there are neurones in LH which are specifically inhibited by it. These neurones are activated by direct applications of insulin, 2-deoxy-D-glucose and 3-O-methyl-D-glucose⁷. Booth⁸ demonstrated that the injection of 1 μ l of 5% D-glucose in the LH blocked insulin-induced eating. More recently Colin-Jones and Himsworth⁹ reported that an injection of 2-deoxy-D-glucose (2DG) into the LH causes a marked increase in gastric acid secretion and attributed it to the lack of metabolisable glucose in the LH neurones. The fact that the specific glucose-sensitive cells which are inhibited by glucose exist only in LH has been highlighted in a recent report in which gastric secretion induced by the peripheral administration of 2DG in the cat was blocked by specific and discrete lesions in the lateral hypothalamus¹⁰. Here we report that the inhibition of LH neurones induced by direct application of glucose is the result of membrane hyperpolarisation and possibly results from the activation of the sodium pump causing an increased extrusion of internal sodium.

Twenty-four male Wistar BR46 rats, fed *ad libitum*, were anaesthetised with intramuscular urethane (1.5 g kg⁻¹). Glass microelectrodes filled with 3 M KCl, d.c. resistance 10–20 M Ω , used for recording single neurone activity, were cemented under a microscope to seven-barrelled micropipettes with α -cyanoacrylate. Intracellular recordings with extracellular glucose application were accomplished by keeping the tip of the multibarrelled pipette 10 to 30 μ m higher than the tip of the recording electrode.

The multibarrelled micropipette (overall tip diameter, 1 μ m) was filled with D-glucose 0.5 M per 0.15 M NaCl, sucrose 0.5 M per 0.15 M NaCl, ouabain 0.017 M, sodium azide 1.5 M, and monosodium L-glutamate 2 M. The d.c. resistance of each barrel ranged from 75 to 500 M Ω . For the application of a particular substance, a constant current of appropriate polarity and strength was passed through the barrel^{11,12}. Criteria laid down by Curtis and Koizumi¹³ were followed to differentiate the effect of the electric current on its own from that of the substance. The injection-recording sites in the LH were A: 5.0–4.2 (from rostral to caudal edges of the VMH); L: 1.5; H: -2.0 to -3.4 after König and Klippel's atlas¹⁴, and confirmed histologically. The methodological details have been described earlier¹¹.

Out of forty-five LH neurones on which glucose was applied, ten increased the frequency of their discharges. This facilitatory effect, however, was also observed on application of Na and sucrose, showing the essentially osmosensitive nature as reported earlier by us^{6,7}. Twenty neurones did not respond to glucose application. The remaining fifteen were specifically inhibited by glucose application but not by Na or sucrose application. Together with our other results, this shows that although the osmosensitive and glucose-sensitive neurones are mingled within the whole LH, the former are more common in the dorsal part and the latter more common in the ventral part of the LH. A typical intracellular record of the activity of a glucose sensitive neurone is given in Fig. 1. It is seen that the application of glucose with a constant current of 10 nA produced marked inhibition on the spontaneous spike generation accompanied by a hyperpolarisation of the mem-

brane by about 5 mV. Moreover, change of the membrane resistance did not occur, as seen in the constant amplitude of the small hyperpolarising pulse near the start of each trace, produced by brief current applied through the bridge-balanced recording electrode. It seems, therefore, that the glucose hyperpolarisation is not due to any change in membrane permeability.

It is known that the extrusion of the intracellular sodium by sodium pump is inhibited by ouabain and by sodium azide^{15–17}. Figure 2a shows a record in which the application of glucose at +30 nA produced a decrease in the extracellular spike discharge with a latency of 10 s and then a complete inhibition at 20 s. Application of ouabain at the dose of -50 nA led to a resumption of spike discharge at the original rate. Glucose, reapplied one min after ouabain application, did not show its specific inhibitory effect. The ouabain antagonisation of glucose-induced inhibition lasted for 2–3 min in general, after which glucose, when applied, again exhibited its characteristic inhibitory effect.

L-Glutamate was applied to relatively quiet glucose-sensitive LH neurones at first, to increase their discharge rate¹⁸, and the effect of ouabain on the glucose-induced inhibition was tested again. As shown in Fig. 2b application of L-glutamate at -30 nA on the glucose-sensitive neurone increased its firing rate from 1–2 to 30–40 Hz. When glucose was applied through another barrel of the pipette at +20 nA, the spike discharge decreased after a latency of 6 s and was completely suppressed in 10 s. A superimposed application of ouabain for 20 s through the third barrel now led to the resumption of the glutamate-induced discharge. It can be noticed that the ouabain effect continued for more than 1 min after its applied current was cut off, and that the glutamate firing was resumed about 10 s after the cessation of glucose current. It was also noticed that whenever ouabain blocked the inhibitory action of glucose, it increased the spontaneous firing. These effects of ouabain were repeatedly demonstrable in four LH neurones (Table 1). The LH neurones are covered by the lamella processes of the astrocytes which are likely to impede the

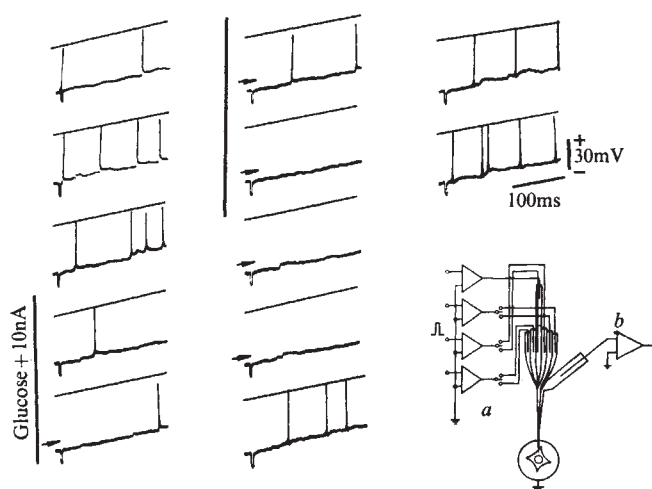


Fig. 1 Intracellular recording from a neurone in the LH depicting the inhibition of spontaneous spike discharge by extracellular glucose application. In each record the upper beam indicates the potential before the electrode penetration. Thick vertical line indicates the duration of glucose application, and the arrows indicate the level of membrane potential before glucose application. The hyperpolarisation was seen in the second record after the start of glucose application and lasted for about 3 s after its termination. The resistance of the membrane did not change because the amplitude of the 10 msec hyperpolarising pulse remained constant throughout. The time elapsed between two successive tracings was 1.3 s. The arrangement of the recording and multibarrelled micropipettes is schematically illustrated below the tracings to the right. a, Constant current amplifier; b, d.c. preamplifier.

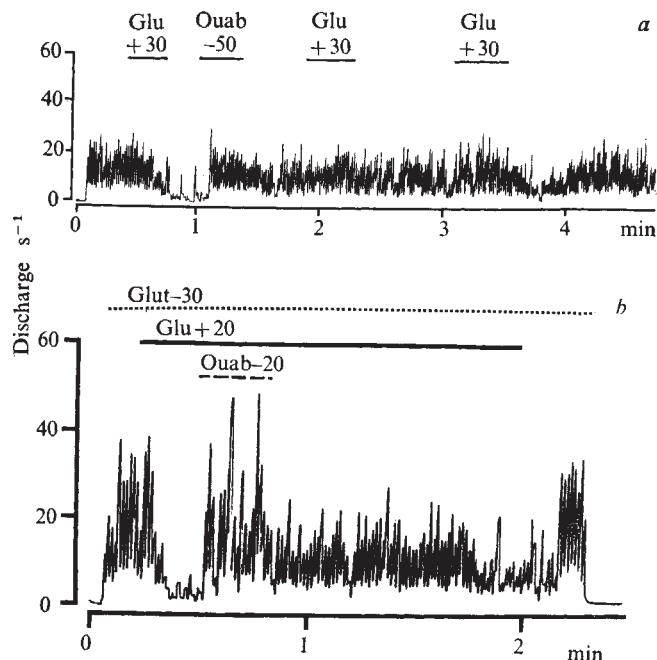


Fig. 2 Pulse counter records of the extracellular spike discharges s^{-1} of two glucose-sensitive LH neurones from two different rats. *a*, Application of glucose inhibited the spike discharge completely. Application of ouabain through another barrel of the same multibarrelled pipette restored it. Further application of glucose showed its inhibitory effect after only about 2 min ouabain application. *b*, Discharge rate of an LH glucose-sensitive neurone was enhanced by the continuous application of glutamate. Application of glucose led to its inhibition but the glucose induced inhibition was antagonised by a superimposed brief application of ouabain. The inhibitory effect of glucose was shown again only a minute or so after the termination of ouabain application. A resumption of vigorous discharge appeared with some latency when glucose application was terminated. Glut, sodium glutamate; Glu, glucose; Ouab, ouabain. The numbers along each abbreviation indicate the current strength (nA) and its direction.

diffusion of chemical injected by microtechnique⁷, and the long latencies of the responses to chemical application could be due to this.

In another five glucose-sensitive neurones the effect of sodium azide was similarly tested, and it was found that glucose-induced inhibition was completely blocked in four of them (Table 1). Thus, the inhibition of these neurones by glucose seems to be caused by a membrane hyperpolarisation which is not due to a permeability change, but is due to the activation of the energy-dependent sodium pump, as it is effectively blocked by cardiac glycosides or anti-metabolites.

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Reciprocal Relationship of Alkaline Phosphatase and 5'-Nucleotidase in Human Bone

THE presence in bone of alkaline phosphatase (APase, EC 3.1.3.1; orthophosphoric monoester phosphohydrolase) has been known for many years^{1,2}. Activity increases during bone growth^{1,3,4} and at the site of Paget's disease^{5,6}, fractures^{7,8}, fibrous dysplasia⁹, and various bone tumours^{5,7,9}. Certain authors have suggested a positive role for APase in bone mineralisation^{1-3,10}. Others have stressed its importance in bone matrix formation¹¹. A third view emphasises the ability of APase to hydrolyse pyrophosphates, thereby relieving their inhibition of bone mineral formation^{12,13}. Dissociation between APase activity and calcification has also been demonstrated¹⁴. The part played by APase in bone growth and mineralisation is thus controversial.

Although 5'-nucleotidase (5Nase, EC 3.1.3.5; 5'-ribonucleotide phosphohydrolase) is more active in bone at physiological pH than APase¹⁵ it has been little studied in this tissue apart from confirmation of its presence in bones and teeth^{16,17} and a report of low activity on histochemical staining of regenerating bone and bone tumours induced in mice⁷. Since changes in 5Nase activity more than that of APase would be expected to alter availability of nucleic acid precursors, the relationship of the two enzymes was examined in bones with intrinsically different growth rates.

Ten femurs and five ribs were obtained from children dying within the first 3 months of life. Samples of the femoral shaft and ribs were obtained from deceased adults. All material was separated within 18 h of death and all specimens were histologically normal. Five samples of osteoblastic bone were obtained *post mortem* from the femoral shaft of patients with Paget's disease.

All samples were cleansed of flesh and marrow. The femoral epiphyses of the neonates were sliced into sections 1-2 mm thick. The remaining bones were crushed. Weighed samples were covered with 10 volumes of water saturated with chloroform and allowed to autolyse for 7 d at room temperature, after which the liquid was decanted, filtered (Whatman No. 4), and centrifuged at 5,000g for 15 min. The APase and 5Nase activity and, where possible, the protein content of the supernatant were measured. APase activity was estimated by assaying the

hydrolysis of 0.1 mM 2'-AMP and 3'-AMP independently in 50 mM Tris HCl containing 10 mM Mg^{2+} , pH 7.9, at 37° C. Adenosine release was monitored by following the decrease in E_{265} which resulted from its deamination by 3.3 µg of adenosine deaminase (Boehringer) added per ml of reaction mixture. 5Nase activity was also measured using 0.1 mM 5'-AMP as substrate, APase hydrolysis of 5'-AMP being competitively inhibited by incorporating 15 mM β -glycerophosphate into the reaction mixture¹⁸.

Protein was measured by precipitating with trichloroacetic acid, washing with organic solvents to remove lipids¹⁹ and redissolving in 0.3 M NaOH before carrying out the Biuret reaction²⁰. Results were expressed as µg protein nitrogen by reference to crystalline bovine serum albumin calibrated by Kjeldahl analysis (Armour Pharmaceutical Co., Eastbourne, Sussex). Enzyme activity as mU per mg protein nitrogen will be referred to as 'specific activity'. Statistical analysis employed the non-parametric U-test²¹ to assess the significance of differences between groups, but the data were organised as mean $\pm$ s.e.m. for presentation. Only differences significant at a level of $P < 0.05$ will be described.

Hydrolysis of 2'-AMP and 3'-AMP as a percentage of the rate of hydrolysis of 5'-AMP by the same preparation, corresponding to the relative proportions of APase and 5Nase in the same sample, is shown in Table 1. There is a striking difference between the femoral shaft in children and in adults. In the former, APase is ten-fold more active than 5Nase, whereas in adults the two enzymes are equally active. The femoral epiphyses, however, are more like the adult bone in respect of the ratio between the two enzymes. This ratio is higher in adult ribs than femur, and lower in neonatal ribs

than femur, so although the ratio in neonatal ribs is somewhat higher than in adult ribs, the difference is not significant. Osteogenic areas of the femoral shaft from patients with Paget's disease have a higher value for this ratio than normal adult femur; in this respect they seem to revert towards the neonatal pattern.

Table 2 presents data expressing the rate of hydrolysis of the three nucleotides per mg protein nitrogen. The 5Nase content of the femoral shaft was significantly greater in adults than in children. Adult ribs had much higher 5Nase activity than neonatal ribs. The 5Nase content of femoral epiphyses was, however, higher than that of neonatal femoral shaft. The 5Nase content of ribs was higher in adults than in infants. The lowest value for 5Nase content among adults was encountered in the osteogenic areas of femur from patients with Paget's disease.

Higher values for APase were seen in the femoral shaft of children and of patients with Paget's disease than in normal adults, but adult ribs had a much higher APase content than infant ribs. Femoral epiphyses had a lower APase content than the shaft in infants.

5Nase and APase are associated with plasma membrane and cytomembrane components in liver and in most cells where their distribution has been examined²². The distribution of 5Nase in bone cells is unknown, but APase is concentrated in microsomal fractions in this tissue²³.

The conditions used in this work were chosen to minimise variability in release and extraction of enzymes. *n*-Butanol increased the APase content of the autolysate but inhibited 5Nase. Deoxycholate increased 5Nase activity more than that of APase and caused dissociation in their rates of release. Autolysis in water at room temperature led to progressive

Table 1 Ratio of the Rate of Hydrolysis of 2'-AMP and 3'-AMP to that of 5'-AMP in Various Categories of Adult and Neonatal Bone

Tissue	Number of specimens	2'-AMP ratio*	3'-AMP ratio*
Epiphyses of femur (children)	10	1.31 $\pm$ 0.35 (0.24–3.57)	1.36 $\pm$ 0.29 (0.17–2.95)
Shaft of femur (children)	9	12.24 $\pm$ 2.46 (4.40–26.40)	11.92 $\pm$ 2.48 (4.80–26.60)
Shaft of femur (adults)	7	0.86 $\pm$ 0.35 (0.01–2.50)	0.96 $\pm$ 0.36 (0.03–2.67)
Paget's disease (femur)	5	3.90 $\pm$ 0.91 (0.88–6.44)	3.75 $\pm$ 1.01 (0.48–6.64)
Ribs (children)	5	4.44 $\pm$ 0.47 (3.43–6.02)	3.55 $\pm$ 0.16 (3.24–4.00)
Ribs (adults)	19	2.63 $\pm$ 1.25 (0.23–7.04)	2.57 $\pm$ 1.13 (0.25–6.48)

*Mean $\pm$ s.e. with range in parentheses.

Table 2 Specific Activities of Nucleotide Hydrolysis of Various Categories of Adult and Neonatal Bone as mU per mg Protein Nitrogen

Tissue	Number of specimens	2'-AMPase*	3'-AMPase*	5'-AMPase*
Epiphyses of femur (children)	5	47.7 $\pm$ 11.7 (14.5–78.1)	75.3 $\pm$ 30.5 (14.5–187.5)	98.4 $\pm$ 50.3 (8.7–290.0)
Shaft of femur (children)	5	174.2 $\pm$ 36.5 (74.4–273.0)	162.2 $\pm$ 30.4 (81.0–245.5)	18.5 $\pm$ 1.6 (15.1–24.5)
Shaft of femur (adults)	6	18.0 $\pm$ 5.5 (1.5–33.0)	25.0 $\pm$ 4.4 (4.5–34.8)	73.1 $\pm$ 24.1 (10.5–161.1)
Paget's disease (femur)	5	167.4 $\pm$ 41.1 (36.6–270.0)	159.3 $\pm$ 40.7 (20.0–245.0)	46.1 $\pm$ 8.2 (18.5–67.5)
Ribs (children)	5	183.0 $\pm$ 28.6 (112.9–251.0)	158.0 $\pm$ 33.4 (63.1–237.5)	45.6 $\pm$ 10.9 (18.7–73.3)
Ribs (adults)	14	618.4 $\pm$ 69.3 (261–1,138)	513.4 $\pm$ 113.4 (170–1,659)	194.6 $\pm$ 75.8 (35–700)

*Mean $\pm$ s.e. with range in parentheses.

increase in specific activity of both enzymes up to 7 d, and the activity ratios were relatively stable between days 5 and 7. Replicate experiments on two different portions of each of five bones showed that reproducibility of the entire procedure for specific activity and enzyme ratios was better than $\pm 12.5\%$.

The physiological role of 5Nase is unknown, but may be related to regulatory control of energy metabolism²⁴ or cell growth²⁵. The following observations suggest that depression of the APase/5Nase ratio may be a mechanism for suppressing bone growth or turnover in long bones: the ratio is lower in adult than in neonatal femur, and is raised when the femur is the site of Paget's disease. Cancellous bone, as exemplified by rib, does not seem to be governed by this mechanism since only slight differences in ratio were noted between neonatal and adult ribs. On the other hand, the specific activity of 5Nase may be a growth-regulatory phenomenon in both types of bone since lower values were found in both ribs and femurs of neonates compared with the corresponding adult bones, while a fall in this activity was noted in Paget's disease.

It is not clear why APase content of adult ribs should be higher than that of neonatal ribs whereas the APase content of the femoral shaft in neonates is much higher than that of the adult bone. This anomaly may reflect differences in the overall metabolic pattern of the two bone types, or in the function of APase in both types. The content and ratio of the two enzymes in the femoral epiphyses differ significantly from those of the shaft in neonates. This might indicate a different role for these enzymes in cell growth as distinct from calcification; or it may reflect differences in the enzyme content of functionally and morphologically different cell types in the various regions of actively growing bone.

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Interaction of Nitrite with Proteins at Gastric pH

THE discovery of volatile N-nitroso compounds¹ in certain foodstuffs² treated with nitrite and/or nitrate has prompted investigation into the conditions likely to give rise to them in food processing and also during digestion. Although other reactions of food components with nitrate and nitrite under physiological conditions are known³, the fate of the total nitrate and nitrite added to foodstuffs as preservatives and that ingested with food has not been established. Our current investigations concern the possibility that interactions of nitrite with certain food components could result in the formation of C-nitroso compounds whose biological behaviour is largely unknown but from the few reports in the literature⁴⁻⁷ are much weaker carcinogens than the corresponding N-nitroso derivatives. The nitrophenols, however, produced by oxidation of the corresponding nitrosophenol, could affect the energy-yielding processes of the cell by uncoupling oxidative phosphorylation⁸.

As part of this study, we have investigated the nitrosation of bovine serum albumin under the conditions of pH and temperature normally recorded in the human stomach. It has been reported⁹ that nitrosation of secondary amines by nitrite occurs in gastric juice, and Philpot and Small⁹ have also shown that nitrosation of tyrosine residues in pepsin occurs at pH 3.8. This paper reports the products obtained from the interaction of nitrite with bovine serum albumin under simulated gastric conditions.

The reaction was carried out in unbuffered solutions, the pH being adjusted initially to 2.5 and allowed to rise during the reaction to 4.0-4.5, thus simulating normal gastric pH changes during digestion. The reaction has been repeated several times and the same results consistently obtained.

Bovine serum albumin (1 g) in dilute hydrochloric acid (10 ml; initial pH 2.5) was treated with sodium nitrite (0.069 g) in a stoppered flask, with stirring at 37° C for 2 h. The resulting yellow solution was immediately dialysed and freeze dried. Weighed amounts of the pale yellow powder were hydrolysed (1) with 6 N hydrochloric acid under nitrogen and (2) enzymatically¹⁰. Acidic and neutral amino acids were analysed automatically by ion-exchange chromatography and peak areas were analysed by computer.

Figure 1 shows the amino acid spectrum from a control sample of albumin, and the additional peaks produced from

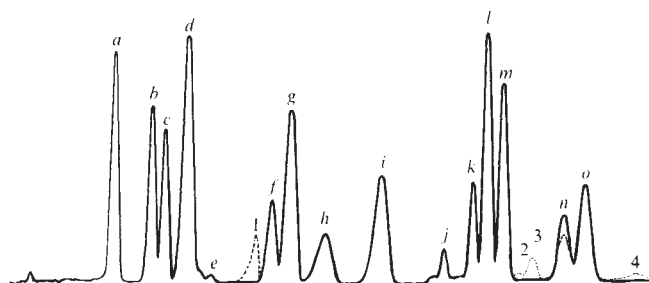


Fig. 1 Amino acids (acidic and neutral) obtained on a Beckman automatic amino acid analyser after hydrolysis of albumin (—, control; ---, after nitrosation). a, Aspartic acid; b, threonine; c, serine; d, glutamic acid; e, proline; f, glycine; g, alanine; h, cysteine; i, valine; j, methionine; k, isoleucine; l, leucine; m, norleucine; n, tyrosine; o, phenylalanine. 1, 6-Hydroxynorleucine; 2, unknown; 3, 3,4-dihydroxyphenylalanine; 4, 3-nitrotyrosine.

the nitrosated albumin after enzymatic hydrolysis; the amino acid spectrum produced from acid hydrolysis was similar to the latter except that peaks 1 and 2 were greatly reduced, almost to an inflection.

On the basis of their elution times by comparison with authentic compounds, peaks 3 and 4 have been identified as 3,4-dihydroxyphenylalanine (dopa) and 3-nitrotyrosine respectively. Their formation could arise by initial nitrosation of available tyrosine residues yielding 3-nitrosotyrosine, which reacts further with the decomposition products of nitrous acid. It is well known that thermal degradation of nitrous acid leads to the production of nitric acid and nitric oxide according to the equation below¹¹.



Therefore 3-nitrotyrosine could arise by nitric acid oxidation of 3-nitrosotyrosine: an analogous reaction is the nitration of phenols by dilute nitric acid which has been shown to be a two-stage mechanism involving initial nitrosation of the activated nucleus followed by nitric acid oxidation to the nitrophenol¹². Alternatively aerial or nitrogen dioxide oxidation of the nitrosotyrosine could give rise to the same product. Whichever mechanism applies all are considered significant in the potential production of 3-nitrotyrosine in the stomach. When the nitrosated protein was hydrolysed with boiling 6 N hydrochloric acid under nitrogen without previous dialysis, residual nitrite caused extensive nitrosation of tyrosine under these more forcing conditions and following oxidation resulted in a large peak for 3-nitrotyrosine with a concomitant decrease (50%) in the tyrosine peak.

The production of dopa can be explained by reaction of 3-nitrosotyrosine with nitric oxide yielding a 3-diazonium nitrate which can be hydrolysed/photolysed to the corresponding hydroxy compound. This sequence of reactions has been demonstrated in the reaction of nitrite with *p*-cresol and the diazo derivative shown to be present by alkaline coupling with β -naphthol¹³.

The presence of both peaks 3 and 4 after enzymatic hydrolysis indicates that they were produced during the reaction period rather than during the vigorous acidic hydrolysis conditions. The ratios of stable amino acids in the control and nitrosated albumins were constant within experimental error with the exception of tyrosine. In the control albumin the phenylalanine: tyrosine ratio was 1:0.68 whereas in the nitrosated albumin this had dropped to 1:0.60. This decrease in tyrosine residues is largely accounted for by the appearance of the dopa residues.

Peak 1 had the same elution time as an authentic sample of 6-hydroxynorleucine¹⁴; this probably arose by deamination of lysine ϵ -amino moieties. Peak 2 has not yet been identified. Loss of peak 1 during acid hydrolysis may have been due to acid-catalysed dehydration to 2-amino-5-hexenoic acid.

To summarise, 3-nitrotyrosine and dopa have been identified from the above reaction and are postulated to be degradation products of 3-nitrosotyrosine, and the ϵ -amino groups of lysine are deaminated to yield 6-hydroxynorleucine concurrently. This series of reactions shows that apart from nitrosamine formation there are other nitrosation reactions involving nitrite in food-stuffs, which could take place under stomach conditions and yield modified amino acids. We are currently investigating the presence of such compounds in cured meats under cooking and digestive conditions.

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Breeding System of a Sessile Animal

Chia and Rostron¹ have suggested that the planula larvae of the common intertidal anemone *Actinia equina* L. are liberated, like those of many other species of anemone, into the plankton. They then enter individuals of the same species, male or female, and are nursed in the coelenteron, eventually being ejected as juveniles of various sizes. Their reasons for suggesting this rather unusual sequence are that almost all of the eighty-five individuals they sectioned were either male or female, and that juveniles occurred impartially in both sexes.

A. equina occurs in a variety of colours, red, brownish red, brown, orange-brown, grass-green, and blue-green, plain or with stripes or dots of yellow or green on the column. The tentacles sometimes differ in colour from the column. I have kept colour varieties in the laboratory, both in mass cultures of a single colour and as isolated individuals, and scored the colours of the juveniles emitted. In general, scoring is easy even for very small young; occasionally small ones, lightly pigmented, may be so distended with water that a dull green pigment in the endoderm becomes visible and modifies the colour of the column, but this is seldom the case when they are contracted. All the juveniles emitted in my tanks that are definitely ascribable to a particular adult have the colour characteristics of that adult even to details, although less intensely pigmented when very small. Equally, all those emitted in mass cultures of a single colour variety are of that variety (Table 1). This was even the case with juveniles emitted by brown adults from Church Bay, Anglesey, although the browns are scattered among a vast preponderance of reds.

To check these results I obtained juveniles from a number of adults *in situ* in a highly variable population at Streedagh, Western Ireland (Table 2). Many individuals yielded nothing, but those that did confirmed the extremely close resemblance of juveniles to their parents (or nurses). A count of adults on the shore gave 226 red, 151 brownish red, 134 brown, 1 orange-brown, and 18 shades of green. The colours of the juveniles obtained from each adult again disagree with a hypothesis of random distribution of larvae into nurses, and with one of random cross-fertilisation.

There are several possible hypotheses to account for this resemblance. (1) It might be that each colour variety is really a separate species. However, sibling species on the shore, unless specialising in different diets, are usually strictly zoned and hardly overlap; this is certainly not the case with the colour varieties of *equina*. (2) The planula larvae might return from the plankton only to individuals of their own colour. Although Chia and Rostron¹ found some planulae

Table 1 Progeny Obtained in Aquaria

Parents		Offspring				
Locality	No.	Red	Red-brown	Brown	Green	
(a) Isolated individuals						
Red						
Church Bay, Anglesey	5	30	—	—	—	
Red-brown						
Plymouth	2	—	7	—	—	
Streedagh, Sligo	8	—	23	—	—	
Brown						
Church Bay	7	—	—	43	—	
Green						
Streedagh	2	—	—	—	9	
(b) Mass cultures						
Red						
Rhosneigr, Anglesey		35	—	—	—	
Church Bay		29	—	—	—	
Brown						
Church Bay		—	—	8	—	

Table 2 Progeny Obtained at Streedagh

Parents	No.	Offspring			
		Red	Red-brown	Brown	Green
Red	12	76	—	—	—
Red-brown	3	—	4	—	—
Brown	3	—	—	7	—
Green	1	—	—	—	2

in plankton samples, they had no success in trying to demonstrate their re-entry into adults. (3) The colour of a juvenile might be determined non-genetically by its nurse. Maternal effects are well known, but a combined foster-mother and foster-father effect would be unique. Also phenotypic maternal effects are not usually persistent, if only affecting colour, for a long period in the juvenile's life. (4) There may be considerable self-fertilisation together with genetic determination of the colours. Chia and Rostron¹ found one hermaphrodite (with both sexes in the same gonad), fifty-four females and thirty males. They noted that only a few mesenteries in an individual are carrying ripe gonads at one time. In so long-lived an animal, without the well-known synchronised outpouring of gametes characteristic of many sessile marine forms, a considerable amount of self-fertilisation could take place during occasional periods when an individual was effectively hermaphroditic, and thus stock itself with juveniles for many months. If the colours are genetically determined, only this hypothesis seems to explain the findings, and work is in progress to test it. The further problem immediately arises of how as many different inbred lines as must occur at Streedagh continue to co-exist on the shore without obvious ecological differentiation.

The colours are known to change if individuals are fed on a diet lacking in carotenoid; all my specimens have been fed on a carotenoid-rich diet and have shown no change over periods, for some of them, of many months. Deprived and decoloured individuals produce young with the same lack of colour². Deprived adults revert to their original colours on an adequate diet³. Sir J. G. Dalyell's pioneering studies⁴ gave much information on the breeding of *equina*. He mentioned explicitly 708 juveniles from five liver-red adults (and he probably saw over a thousand), all of which at emission were red, pink, or, in a few cases, nearly white. Of an unspecified number produced by the bright vermilion variety, all were bright red; one turned green at 5 months and produced more than 103 progeny, all green like itself.

From the constancy of colour in all the others (except one very aged individual), it is unlikely that this one changed by deprivation. This is the only instance in the literature of such a change and must surely be genetic, either a mutation or a stray cross-fertilisation by a sperm from another individual also carrying green as a recessive. Dalyell⁴ saw only liver-reds and vermilions in his populations in the Firth of Forth. Chia and Rostron¹ give no information on the colours of their specimens, but do mention that all the planulae were pinkish or orange. It is possible that red varieties form nearly the whole population on the east coast of Great Britain, and the problem of inheritance of colour varieties was not brought to their attention.

If the hypothesis of a high degree of self-fertilisation is correct, it will give an example of convergent similarity in breeding system and ecogenetics between a sessile animal and many plants. Sessile animals should provide interesting material for such a comparison.

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Blue-Green Bacteria synthesise L-Tyrosine by the Pretyrosine Pathway

THE similarity of major biochemical pathways throughout the animal and plant kingdoms¹ presumably reflects the ancient formation of the pathways in a common ancestral counterpart of modern cell types. The pathway previously known for L-tyrosine biosynthesis in both prokaryotic and eukaryotic microorganisms (Fig. 1a) involves a dehydrogenation-decarboxylation conversion of prephenate to 4-hydroxy-phenylpyruvate followed by transamination in the presence of a suitable amino donor. Our data (Fig. 1b) demonstrate a reversed order of these two reactions in several species of blue-green algae (more appropriately designated bacteria (order Chroococales)).

Comparing the regulation of aromatic amino acid synthesis in *Agmenellum quadruplicatum*² and *Anacystis nidulans* (Kratz-Myers strain), we found that most of the key enzymes were stable. Anthranilate synthetase, 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthetase, prephenate dehydratase, chorismate mutase and an aromatic transaminase all had relatively high specific activities. In contrast, activity for prephenate dehydrogenase, the enzyme expected to catalyse the first reaction unique to L-tyrosine biosynthesis, was not detected. Enzymes able to catalyse the two reactions shown in Fig. 1b have been partially purified from crude, cell-free extracts. The amino acid intermediate, β -(1-carboxy-4-hydroxy-2,5-cyclohexadien-1-yl) alanine, hereafter denoted 'pretyrosine', has been prepared enzymatically (legend, Fig. 1).

A. quadruplicatum, the most intensively studied of the species in our collection, provided most of the evidence for the pretyrosine pathway of L-tyrosine synthesis. We assayed for prephenate dehydrogenase in crude and partially purified enzyme preparations (Sephadex G-200 eluate fractions) at temperatures between 30° and 45° C (2° C intervals) over a

pH range of 6.5–9.0 (0.5 pH unit intervals). Wide variations in ionic strength, the presence of amino acids, or the use of stabilising compounds (such as glycerol or β -mercaptoethanol) revealed no detectable activity. Although described prephenate dehydrogenase activities are NAD^+ -dependent, we also tested for a possible NADP^+ -dependent activity with negative results.

A unique route for L-tyrosine synthesis was suggested, however, by a transaminase enzyme reactive with prephenate as an amino acceptor. The enzyme was partially purified by Sephadex G-200 gel filtration giving a single eluate peak of activity that could utilise prephenate efficiently. Amino donor specificities were characterised by thin-layer chromatography. Transamination of prephenate was confirmed by the appearance of a new ninhydrin-positive spot after thin-layer chromatography. L-Leucine, L-isoleucine and L-phenylalanine were the best amino donors for transamination. Table 1 shows that when L-phenylalanine was the amino donor, prephenate was one of the best substrate reactants.

Table 1 Keto Acid Specificity of Prephenate Transaminase

Keto acid substrate	Specific activity
α -Ketoglutarate	10.4
Prephenate	8.5
α -Keto- β -methylvalerate	5.5
α -Ketoisovalerate	3.1
α -Ketobutyrate	0.9
Pyruvate	0.4
Oxalacetate	0

The reaction mixtures consisted of 0.1 ml of 40 mM L-phenylalanine (amino donor), 0.1 ml of 10 mM keto acid, 0.05 ml of 1 mM PLP, and 0.05 ml of partially purified enzyme (132 μg protein). The reaction was terminated after 20 min of incubation at 45° C by addition of 0.9 ml of 2.67 N NaOH, and the absorbance of phenylpyruvate formed was measured at 320 nm. Specific activity is expressed as $\text{nmol min}^{-1} \text{mg}^{-1}$. Prephenate was prepared by the method of Gibson⁴.

Thin-layer chromatography of the transamination reaction between L-phenylalanine and prephenate gave two ninhydrin-positive spots; one had the same R_F as L-phenylalanine (the standard), and the other was presumably the transamination product of prephenate, that is, pretyrosine. Prephenate is quantitatively converted to phenylpyruvate in 10 min at acid pH (0.1 N HCl) by a well documented dehydration-decarboxylation reaction⁵. Likewise, if our proposed structure for pretyrosine (Fig. 1) is correct, it would be converted to L-phenylalanine at acid pH. The product of acid-treated pretyrosine migrated at an R_F identical to that of L-phenylalanine after thin-layer chromatography. To confirm this identity, a reaction mixture of prephenate, L-leucine, and partially purified transaminase was incubated for 60 min at 45° C. The protein was discarded after acid treatment, and the supernatant was analysed using a Beckman model 120-C amino acid analyser. Apart from the L-leucine initially present as amino donor, only L-phenylalanine was detected.

The second reaction of L-tyrosine biosynthesis in *A. quadruplicatum* is the dehydrogenation of pretyrosine. An active enzyme able to catalyse the reaction was isolated by Sephadex G-200 gel filtration. (The preparation of pretyrosine is described in the legend to Fig. 1.) The reaction was confirmed using an assay mixture of 0.1 ml of 5 mM NAD^+ , 0.1 ml of partially purified pretyrosine dehydrogenase and 0.1 ml of 5 mM pretyrosine. The following confirmatory determinations were made. (i) Disappearance of pretyrosine: after 90 min of reaction at 37° C, 10 μl was spotted on a cellulose plate (as described above); a pretyrosine spot was barely detectable. (ii) The continuous formation of NADH: NADH was measured fluorometrically at 37° C with an Aminco Bowman spectrophotofluorometer using an excitation wavelength of 340 nm and an emission wavelength of 460 nm. The initial rate of formation was rapid when the above reac-

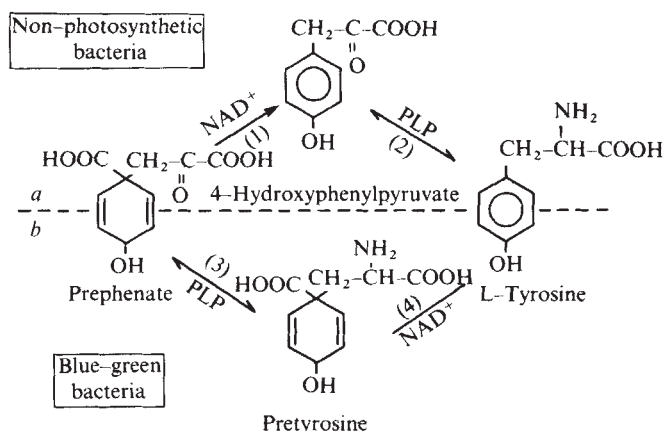


Fig. 1 Biosynthesis of L-tyrosine in prokaryotes. *a*, The 4-hydroxyphenylpyruvate pathway; *b*, the pretyrosine pathway. Abbreviations: PLP, pyridoxal-5-phosphate; NAD^+ , nicotinamide adenine dinucleotide. Enzyme denotations: (1) prephenate dehydrogenase; (2) 4-hydroxyphenylpyruvate transaminase; (3) prephenate transaminase; and (4) pretyrosine dehydrogenase. Pretyrosine for use as substrate was prepared as follows. A reaction mixture of 7.2 mM prephenate, 20 mM L-leucine, partially purified transaminase from *A. quadruplicatum* and 10 mM Tris buffer, pH 8.5, in a volume of 20 ml was incubated for 90 min at 45° C. After removal of protein by passage through an Amicon Diaflow cell (PM 10 filter) the reaction mixture was applied to a Dowex 21K column in the Cl^- form which had been equilibrated with 10 mM ammonium formate, pH 7.5. The column was washed with approximately 30 ml of the 10 mM ammonium formate until no L-leucine could be detected in the effluent by thin-layer chromatography. Pretyrosine was eluted with 0.3 M ammonium formate, pH 7.5, and prephenate remained bound to the column. Fractions containing pretyrosine (located by thin-layer chromatography) were pooled. Further purification attempts led to significant recovery losses owing to the chemical lability of pretyrosine. Because it was stable when stored frozen in 0.3 M ammonium formate, it was used in this form. The concentration of pretyrosine was approximately 5 mM (determined by acid conversion to L-phenylalanine followed by fluorometric analysis of L-phenylalanine concentration (*Sigma tech. Bulletin No. 60F*)).

tion mixture was used (specific activity, 40.9 $\text{nmol min}^{-1} \text{mg}^{-1}$). (iii) Appearance of L-tyrosine: after 90 min at 37° C fluorometric assay⁶ of the reaction mixture indicated 100% conversion of pretyrosine to L-tyrosine, while amino acid analysis verified that L-tyrosine was the only amino acid detectable.

Although major biochemical pathways in microorganisms commonly involve an identical sequence of steps, a diversity of regulatory patterns has evolved⁷. Thus in *A. quadruplicatum* with its different pathway, novel aspects of regulation are not unexpected. The activity of prephenate dehydratase (Fig. 2) is controlled by both L-phenylalanine and L-tyrosine. L-Phenylalanine (1 mM) causes 90% inhibition, while L-tyrosine at the same concentration stimulates activity about two-fold. The results of our *in vitro* transaminase enzymology indicate that a single transaminase catalyses both the phenylpyruvate transaminase reaction (denoted (2) in Fig. 2) and the prephenate transaminase (2') reaction.

The regulatory pattern depicted in Fig. 2 can be summarised as follows. In panel (a) where L-tyrosine is present in excess, prephenate dehydratase possesses an advantage in its competition with prephenate transaminase for prephenate. This, in conjunction with the increased supply of phenylpyruvate, leads the transaminase to function primarily as phenylpyruvate transaminase. Hence, in the presence of L-tyrosine, metabolite flow is pivoted toward L-phenylalanine synthesis. In Fig. 2b L-phenylalanine is assumed to be present in excess, and the regulatory features that tend to direct metabolite flow toward L-tyrosine are illustrated. Prephenate is shunted toward L-tyrosine synthesis owing to (i) feedback inhibition of prephenate dehydratase, and (ii) the effectiveness of L-

phenylalanine as an amino donor in the prephenate transaminase reaction. These regulatory relationships appear well suited to maintaining balanced intracellular levels of L-tyrosine and L-phenylalanine.

In addition to *A. quadruplicatum* and *A. nidulans*, pretyrosine dehydrogenase activity (but no prephenate dehydrogenase activity) was verified in crude extracts prepared from four other species (obtained from L. O. Ingram): *Coccochloris elabens* strain 17A³ (unicellular, marine); *Nostoc muscorum* strain Mac⁹ (filamentous, freshwater); *Lyngbya lagerheimii* strain Mont¹⁰ (filamentous, marine); and *Oscillatoria* sp. strain SFO (filamentous, marine; isolated by Ellen Brandt). Partial purification will be required to confirm the expected presence of prephenate transaminase in these species.

Blue-green bacteria and other prokaryotes are thought to have diverged from a common ancestor 3–4 × 10⁹ yr ago¹¹. If

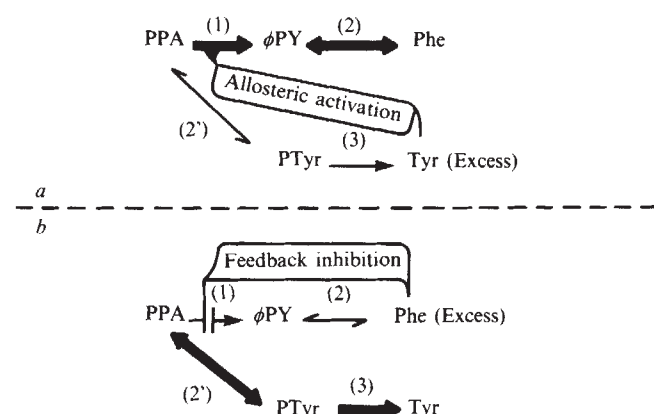


Fig. 2 Regulation of L-tyrosine and L-phenylalanine biosynthesis in *A. quadruplicatum*. *a*, L-Tyrosine is present in excess and L-phenylalanine is limiting. *b*, L-Phenylalanine is present in excess and L-tyrosine is limiting. Heavy arrows denote the metabolite shift toward L-phenylalanine synthesis in the presence of excess L-tyrosine (*a*) and toward L-tyrosine in the presence of excess L-phenylalanine (*b*). (1) Prephenate dehydratase, (2) phenylpyruvate transaminase; (2') prephenate transaminase; (3) pretyrosine dehydrogenase. Prephenate dehydratase activity was assayed as described by Jensen⁸, except that 50 mM Tris buffer, pH 7.8, was used.

further comparative biochemistry is consistent with our findings, it would provide significant data that might help to clarify the evolutionary sequence. It is commonly speculated that the mitochondrial and chloroplast organelles of eukaryotes are endosymbiotic descendants of ancient prokaryotes. A logical corollary of this hypothesis is that the ancient origin of mitochondria and chloroplasts can be identified with non-photosynthetic and blue-green prokaryotes, respectively. The possibility that blue-green bacteria can be clearly distinguished from other prokaryotes by L-tyrosine pathway enzymology should provide a direct test of this evolutionary hypothesis.

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Electron Microscopic Visualisation of Chromosomes banded with Trypsin

CHROMOSOME banding methods have been widely used to identify normal and rearranged chromosomes^{1–11} and to probe the structural and biochemical organisation of chromosomes^{11–13}. The methods most commonly used include Q^{1,2}, G^{3–6}, C⁷ and R banding⁸, each producing characteristic bands on metaphase chromosomes as observed by light microscopy. Attempts to observe banding at an ultrastructural level have not been particularly successful^{12,14,15}, but since such studies could provide more detailed maps of chromosomes and help to clarify the structural nature of the bands, further attempts were made in my laboratory to develop a reliable and simple method for observing chromosome bands at the electron microscope level, using trypsin as the banding agent⁵.

Log phase Chinese hamster Don cells (American Type Culture Collection, Rockville, Maryland) were accumulated at metaphase by 4 h of incubation with colcemid (0.05 µg ml⁻¹) in McCoy's medium 5A + 10% foetal calf serum. Metaphase cells were then dislodged into the medium by shaking, collected by centrifugation, treated with 0.075 M KCl for 15 min at 37° C, and fixed for 60 min with three changes of fixative (one part glacial acetic acid : three parts methanol). Formvar films (0.5% formvar in ethylene dichloride) were made on glass microscope slides and then coated with carbon. Each coated slide was slowly lowered into a shallow trough of water so that the film detached and floated on the surface of the water. A piece of wire gauze was placed on the bottom of the trough, and 75 mesh electron microscope grids were arranged on top of the gauze. Just before use, the fixed cells were suspended in a mixture of one part glacial acetic acid and six parts methanol, that is, the concentration of acetic acid in the original fixative was reduced. A drop of this cell suspension was placed on top of the floating film and air-dried. The wire gauze, holding the grids, was then raised out of the water so that the film, supporting the cells, was spread smoothly over the grids. After drying at room temperature, individual coated grids were removed from the gauze and checked with a phase contrast microscope to ensure that well-spread chromosome preparations were present.

When fixed cells are spread on formvar, or formvar-carbon films attached to glass slides (glass substrate), the acetic acid in the fixative disintegrates the film. This problem has been circumvented by using water as a substrate for the film, since water protects the formvar from the fixative in some unknown manner. The reduction in the acid content of the fixative just before spreading the cells seems to safeguard the film further. These precautions render the film impervious to the fixative, and high quality metaphase preparations can be made.

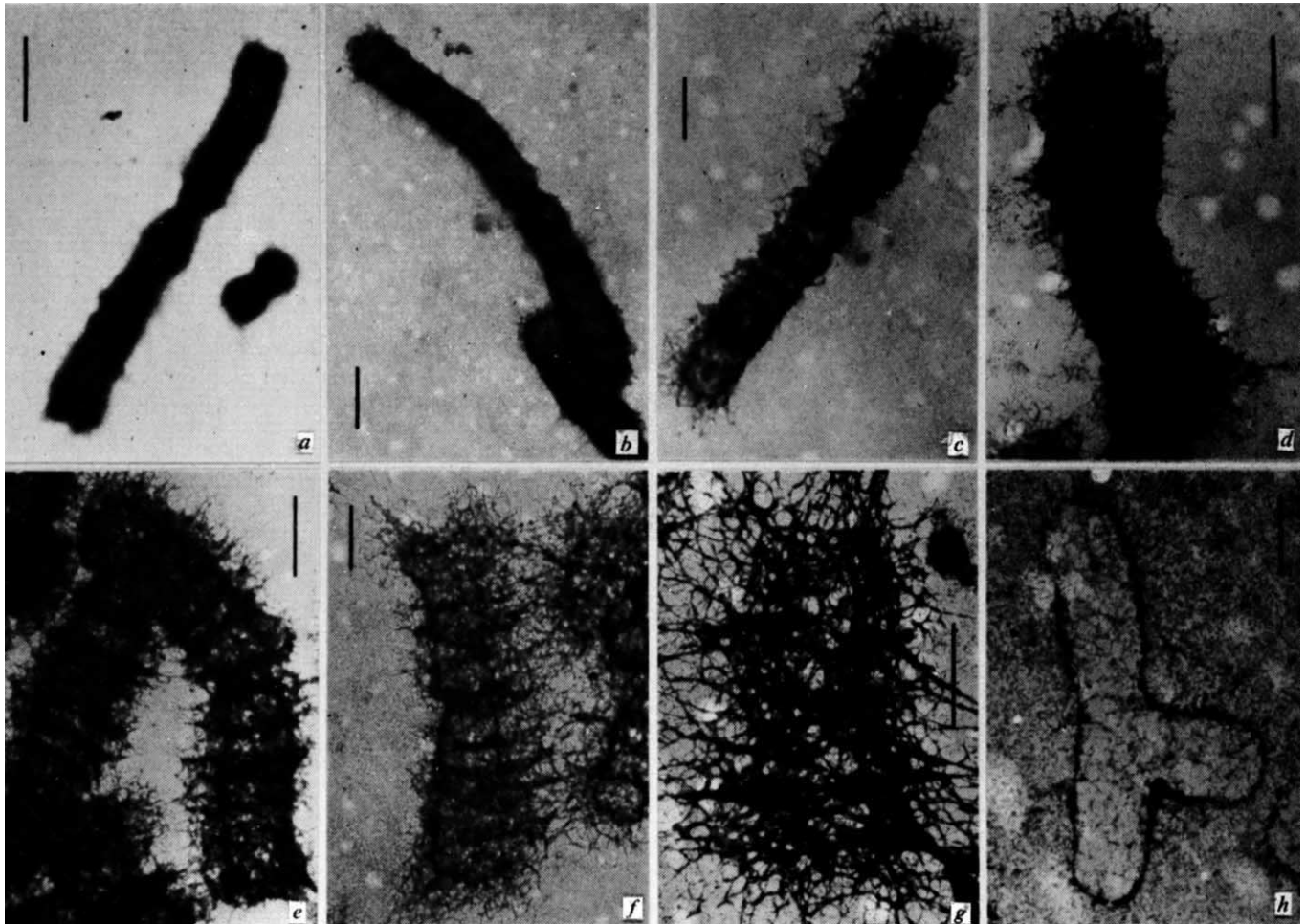


Fig. 1 The bars represent 2 μ m. *a*, Untreated (control) Chinese hamster chromosomes, stained with uranyl acetate. *b*, Banded chromosome, treated with trypsin for 1.5 min, unstained. *c* and *d*, Chromosomes treated with trypsin for 2 min (*c*) and 1.25 min (*d*), unstained. *e* to *g*, Chromosomes treated with trypsin for 1.5 min (*e*) and 2 min (*f* and *g*); *f* is unstained; *e* and *g* are stained with uranyl acetate. *h*, Two overlapping chromosome 'ghosts'. Trypsin treatment lasted 10 min; Giemsa stain. Residual chromatin fibres can be seen.

For G banding, grids were floated face down on 0.1% trypsin (Difco), made in Ca^{2+} -free and Mg^{2+} -free balanced salt solution, for 1 to 10 min. Each grid was rinsed in two changes of 70% ethanol and two of 100% ethanol, and air dried. The extent of banding was monitored by placing the grid on a glass slide and examining by phase contrast microscopy.

This electron microscopy method of chromosome preparation and G banding is the same as for light microscopy, and allows comparisons of the same chromosome to be made at both levels of resolution. For electron microscopy, trypsin-treated or untreated (control) chromosome preparations were: (1) left unstained, (2) stained with Giemsa for 5 min, or (3) stained with 2% uranyl acetate for 10 min, dehydrated through a graded series of ethanol washes and rinsed in amyl acetate for 20 min before air-drying. Specimens were examined with a Philips 200 electron microscope, operating at 60 kV.

Untreated chromosomes stained with uranyl acetate or Giemsa appeared to be very electron-dense structures at the ultrastructural level (Fig. 1*a*). The chromosome morphology was well preserved. Some of the preparations stained with uranyl acetate suggested a hint of lateral striations along the chromosomes, but the Giemsa-stained chromosomes were particularly dense in appearance, and showed no fine detail. Chromatin fibres, like those seen in whole mount preparations^{16,17}, were not apparent in untreated chromosomes,

suggesting that these fibres had all become melded together, presumably as a result of the fixative. This, however, proved not to be the case when the trypsin-treated chromosomes were examined.

Bands were readily apparent in trypsin-treated chromosomes regardless of whether the chromosomes were stained with uranyl acetate, or Giemsa, or were left unstained. At low magnifications, complete chromosome complements were observed (Fig. 2). Depending on the extent of trypsin digestion, the chromosomes exhibited different degrees of disruption. In mildly treated chromosomes, bands appeared as electron dense areas, while interband regions were much less dense (Fig. 1*b*). Preliminary results suggest that the electron dense bands correspond to the G bands observed by light microscopy. Some of the banded chromosomes showed little or no dispersion of the chromatin fibres (Fig. 1*b*); however, in many others, chromatin fibres appeared to spew out of the chromosome, particularly from interband regions (Fig. 1*c* and *d*). More severely treated chromosomes showed an extensive dispersion of chromatin fibres, apparently affecting both bands and interbands (Fig. 1*e* to *g*); however, even in severely distorted chromosomes, the bands could still be identified as such (Fig. 1*f* and *g*), suggesting that the packed or folded chromatin in the band regions was resistant to the dispersive effect of the trypsin. Completely overdigested chromosomes resembled ghosts; however, a few remaining chromatin fibres could still be identified (Fig. 1*h*).

In some trypsinised preparations, chromatin fibres could be seen very clearly (Fig. 1*g*), and measurements indicated that many were comparable in diameter (about 250 Å) to those found by whole mount electron microscopy^{16,17}. The methods of chromosome preparation (hypotonic, fixation) and trypsinisation do not seem adversely to affect the chromatin fibres, at least as far as dimensions are concerned.

These results indicate that fine structural studies of G

banded chromosomes are now feasible. There may be many previously unidentifiable structural details in chromosomes which could be particularly useful in cytogenetic studies, and the high degree of resolution offered by this method should permit the construction of more detailed chromosome maps

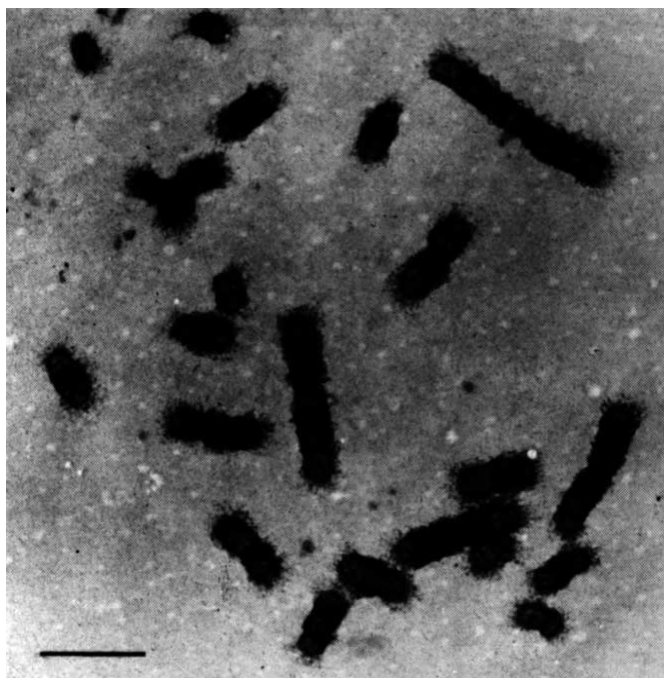


Fig. 2 Electron micrograph of a complete Chinese hamster chromosome complement, treated with trypsin for 2 min. Unstained preparation; the bar represents 5 μ m.

which could be of considerable value in pinpointing the precise location of structural rearrangements.

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What Abrogates Heart Transplant Rejection in Immunological Enhancement?

IMMUNOLOGICAL enhancement is historically the oldest method for impeding graft rejection. Such an outcome resulting from the injection of antiserum directed against a transplant seemed paradoxical, and the mechanism has been under discussion all through the years of study of antigens as putative tolerogens to the present time when immunological enhancement is the best available method for donor-specific immunosuppression in animal models¹. The shortcomings of non-specific immunosuppression at present used in clinical transplantation are so great that an interpretation of the mechanism of immunological enhancement is important, in order to find how the method can best be adapted for clinical use.

That antibodies against gene products of the major histocompatibility genetic complex (MHC) are responsible for mediating passive enhancement seems compelling from studies in which congenic strains of mice have been used. This was interpreted in the sense that in mice, H-2 antigens, the much studied serological markers for the mouse MHC, and their antibodies, played the key role. Any such conclusions would apply equally to HL-A in man, Ag-B (H-1) in the rat, and so on, because these are genetically homologous chromosomal regions.

Enquiry into the mechanisms of passive enhancement has mainly followed two courses: studies of the class of anti-

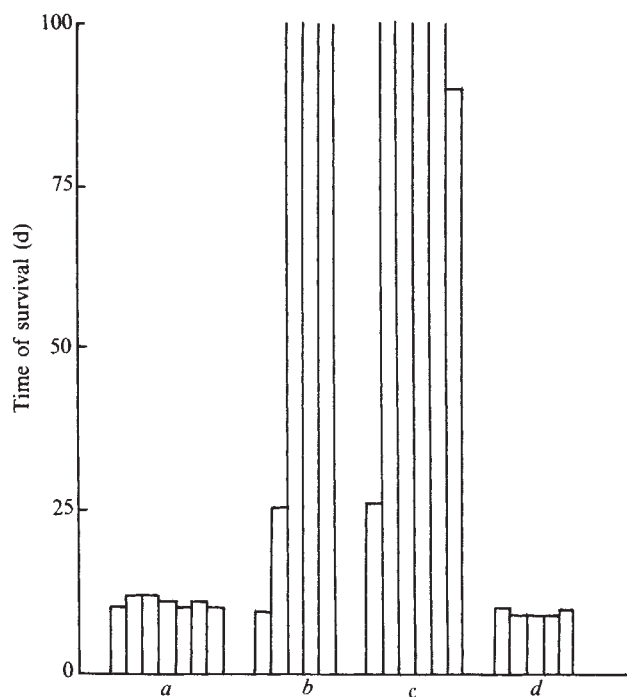


Fig. 1 Survival of rat auxiliary heart transplants from (A/gus $\times$ Wag) F₁ to A/gus. Series a, controls injected with normal rat serum, 1 ml (intravenous) on day of grafting and days 1, 3, 5, 7, 9 thereafter. Series b, injected (the same dose schedule) with enhancing alloantiserum; series c, treated with the same serum but fully absorbed with Wag erythrocytes; series d, the same serum fully absorbed with Wag erythrocytes and subsequently absorbed with Wag spleen/thymus cells.

body involved², and of the mechanisms by which they act (peripheral, afferent or central blocks). Recent work with enhanced rat kidney allografts shows some unbound antigen in the grafted tissue³ and will focus attention on the favoured 'central block' hypothesis. This is based on the idea that in passive enhancement, antibody binds to transplantation antigens from the graft and the complex blocks the lymphocyte recognition site because this site is specifically related conformationally to that antigen. There is much supporting evidence for this⁴ but unfortunately the nature of the T lymphocyte recognition site is not known. Whether it is an IgX or not, Ir (immune responsiveness) genes located in the MHC

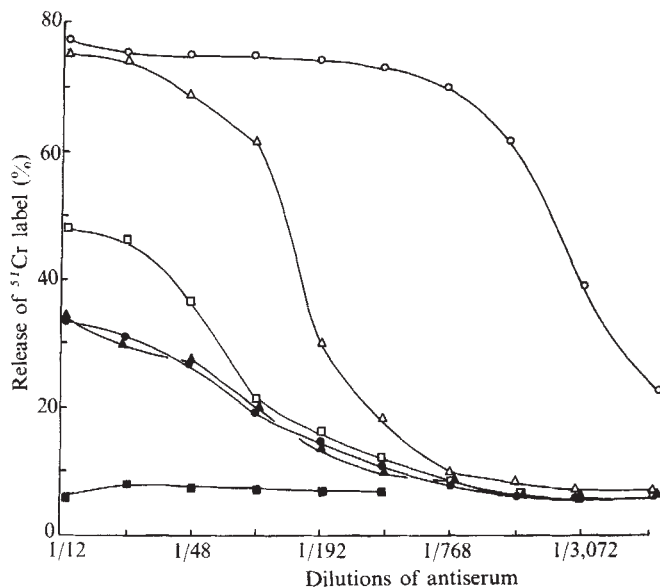


Fig. 2 Absorption of *A/gus* anti-(*Wag* × *A/gus*) F_1 alloantiserum with *Wag* erythrocytes. Complement-mediated cytotoxicity using *Wag* lymph node target cells, measured by ^{51}Cr release. The original titre, $\circ$ — $\circ$; after absorption with erythrocytes once, $\triangle$ — $\triangle$; twice, $\square$ — $\square$; three times, $\bullet$ — $\bullet$; four times, $\blacktriangle$ — $\blacktriangle$; subsequent absorption with spleen cells, $\blacksquare$ — $\blacksquare$.

chromosomal region are involved with antigen recognition in some as yet undefined way. Different biological roles have been attributed to various segments of the MHC (ref. 5) and it has been suggested that the MHC associated Ir gene product might be the T cell recognition site⁶.

In the experiment described below the question asked is not what class of MHC-directed alloantibody is involved but what is its specificity? It tests the proposition that histocompatibility antibody and hence transplantation antigens as detected by 'tissue typing' may have little to do with transplantation. During the past year evidence has been presented that mixed lymphocyte reactions (MLR)⁷ and graft-versus-host reactions (GvH)^{8,9} may occur in the absence of serological differences in the mouse H-2 system.

The model we have used is that of heterotopic (abdominal) auxiliary heart transplants, anastomosing aorta to aorta and pulmonary artery to inferior vena cava. The method was modified from Ono and Lindsey¹⁰. The endpoint for rejection was cessation of heart beat, which was somewhat sharper than ECG recordings. Two inbred lines of rats were used which differed at their MHC, *A/gus* (Ag-B.1= $H-1^1$) and *Wag* (Ag-B.2= $H-1^w$). In the experiments quoted (*A/gus* × *Wag*) F_1 female hearts were transplanted to *A/gus* male recipients. Alloantisera against *Wag* rats were raised in *A/gus* rats by immunisation with (*A/gus* × *Wag*) F_1 spleen cells; batches of rats were injected weekly eight times with spleen cells amounting to 10^7 leukocytes at each injection. Such sera generally had cytotoxic titres against *Wag* lympho-

cytes (^{51}Cr release complement-mediated cytotoxicity) of about 1/1,000. The sera consistently led to prolonged and usually indefinite survival of heart transplants when given intravenously in six doses of 1 ml each over the first 9 d after grafting. Less than this amount gave irregular results. This situation is illustrated as series *b*, Fig. 1, to be compared with series *a* (normal serum treated controls) which rejected their transplants between 8 and 11 d. Some of the transplants in series *b* and *c* have now survived for more than 250 d. These results agree well with those previously described using this combination of strains¹¹.

Enhancing serum as used in series *b* was then absorbed to remove Ag-B antibodies but possibly not antibodies against (? serologically silent) products of other genes in the MHC, if such exist. Absorption was monitored by loss of Ag-B cytotoxic titre but we assume absorption is not immunoglobulin-class discriminative and that any non-cytotoxic antibody of the same Ag-B specificity is removed at the same time. There is no precedent for the alternative assumption of immunoglobulin class discrimination in absorption. The source of material for such an absorption is not obvious, but in rodents, erythrocytes carry histocompatibility antigens and there are reasons to suppose that they might not carry other gene products of the MHC (they express no MLR or GvH reactivity). Rat red cells were purified from blood using a closely packed cotton wool column more or less as described by Diepenhost *et al.*¹². *Wag* blood (500 ml plus anticoagulant) was centrifuged and the cells resuspended in 500 ml of equal volumes of the supernatant plus saline; this was passed through a column 4 cm × 60 cm at 2 pounds per square inch. Fractions were pooled up to two tubes before the first showing any white cells. The purified red cells from one column were sufficient to absorb 100 ml of alloantiserum in three stages until negligible cytotoxicity remained which could not be reduced by further absorption. The absorbed sera were centrifuged at 105,000g for 1 h to remove debris before use. Cytotoxicity tests on absorbed and unabsorbed sera are shown in Fig. 2. This red-cell-absorbed serum, used in the same doses as in series *b*, enhanced the transplants at least as well as the original serum (Fig. 1, series *c*).

Cells used for absorption are generally presumed to carry away the bound antibody; it is not obvious, therefore, that soluble Ag-B antigen/antibody complexes would be left in the serum. This is, however, an important point in the interpretation of the results and was checked as follows. Complexes were sought directly by purifying rat IgG (by DEAE ion-exchange chromatography), labelling with ^{125}I and adding this as a marker to a sample of the red cell absorbed enhancing alloantiserum. This mixture was examined on a Sephadex G200 column (Fig. 3) and monitored for ^{125}I γ counts and by rabbit immunoglobulin anti-rat IgG in immunodiffusion assays. The ^{125}I counts located rat immunoglobulin on the column whereas immunodiffusion

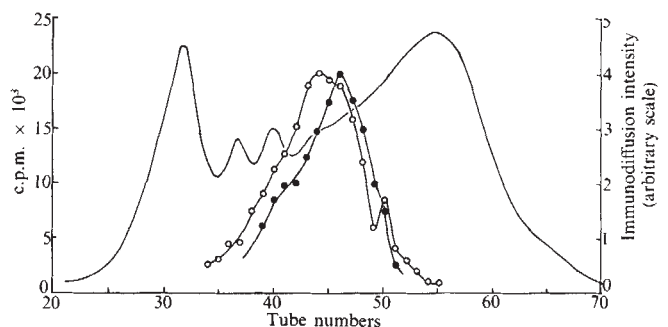


Fig. 3 Sephadex G200 column gel filtration of erythrocyte-absorbed enhancing alloantiserum. Protein profile (280 nm absorption), uninterrupted line. ^{125}I -labelled purified rat IgG added as marker, $\circ$ — $\circ$. Rat immunoglobulin as tested by a rabbit IgG anti-rat IgG using immunodiffusion, $\bullet$ — $\bullet$.

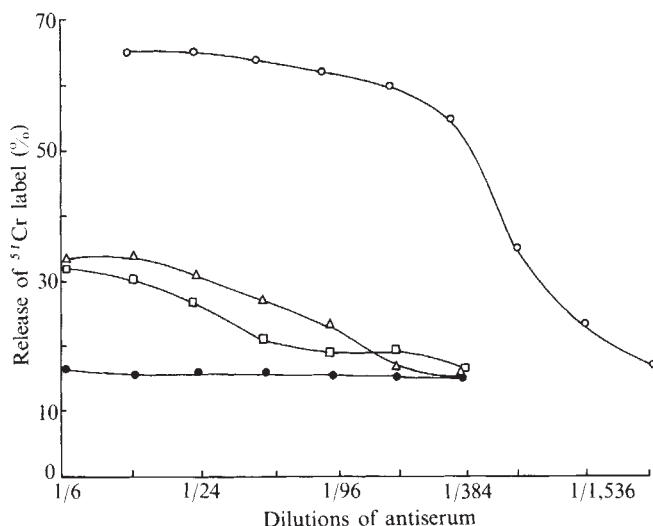


Fig. 4 Complement-mediated cytotoxicity of enhancing alloantiserum. Original titre against *Wag* lymph node target cells, ○—○. After maximal absorption with *Wag* erythrocytes, titre against spleen cells, △—△; against lymph node cells, □—□. There was no measurable reaction (●—●) using as target cell, bone marrow, thymocytes, peripheral leukocytes (unfractionated) or normal peritoneal exudate cells.

with anti-IgG was expected to locate both free and antigen-bound immunoglobulin. The two peaks showed no differences attributable to antigen-antibody complexes. This method possibly lacks the required sensitivity but shows at least no gross contamination with complexes. Further information was sought by taking red-cell-absorbed enhancing serum (the same batch as used in series c, Fig. 1) and reabsorbing with white cells (mixed spleen/thymus used at 5×10^8 cells per ml serum). This treatment would remove antibody of any specificity still present because spleen cells were used for the original immunisation, but their ability to remove complexes by binding the Fc portion of complexed immunoglobulin would presumably be more modest. As can be seen in Fig. 1, series d, this doubly absorbed serum had no enhancing activity. These facts support the idea that if complexes are present owing to shedding from erythrocytes they are in small amounts. Because less than 6×1 ml of original enhancing antiserum was inadequate to give consistent permanent heart allograft acceptance, the absorbed serum should not have been effective with its (at least) greatly reduced Ag-B (complexed) antibody content when used at the same dosage, if the transplant protection was Ag-B mediated.

We therefore conclude that Ag-B antibody is not the essential mediator of immunological enhancement, either alone or as a complex, and hence that some antibody of different specificity must be involved. It is possible to imagine that heart allografts in rats are not affected by Ag-B histoincompatibilities or not affected by the Ag-B 1/2 incompatibility in particular. Then rejection could be attributed to multiple non-Ag-B differences. This is unprecedented, which is no good reason for rejecting the idea, but aural/foetal heart allografts in mice obey H-2 rules so we do not give serious consideration to this suggestion. We do not believe that heart-specific alloantigenicity is involved because the enhancing alloantiserum was raised with immunogen which would not carry any such specificity. We suggest that the effective antibody in the absorbed serum is directed against T cell receptor sites and may be an antibody against rat Ir-1^w. We favour the view that Ir-1^w gene product from passenger (and resident) leukocytes provides the antigen to complex with the antibody which then blocks the graft recipient's lymphocyte recognition molecules. The implication is that transplant sensitisation is due to donor

lymphocytes in the transplanted tissues and any other cells expressing MHC associated gene products. This presumably excludes heart tissue itself.

The residual cytotoxic titre remaining for lymph node cells in red-cell-absorbed enhancing serum (Fig. 2) which cannot be removed by further red cell absorptions and was referred to as negligible is, of course, not negligible. It might be Ag-B antibody for which red cells lack the Ag-B specificity.

The results illustrated in Fig. 4 weigh against such an interpretation; the residual titre can be detected using lymph node cells or spleen cells as labelled target but not using thymocytes, peripheral blood leukocytes, bone marrow cells or normal peritoneal exudate cells. Neither liver nor blood platelets would absorb it out. This is not the cell-type distribution seen for H-2 in the mouse or HL-A in man. Liver is the most effective source of material for absorption of H-2 and HL-A antibodies. This weak cytotoxic effect might not be connected with the enhancing properties of the sera, but if it is, then lack of reactivity by naive thymic lymphocytes is interesting; where might their recognition sites be sequestered until they have escaped thymic influence and been educated?

The serological properties of this absorbed serum are remarkably similar to those described for a proposed Ir-1.1 serum in the mouse¹³. This serum was obtained by cross immunisation of two congenic lines derived from a recombinant such that the only genetic differences lay between *H-2K* and *Ss-Slp*. We propose that absorption with suitable material (for example, red cells, liver, platelets) can also provide a similar class of antiserum, directed at MHC-associated gene products other than those hitherto detected by tissue typing. The role we now ascribe to such antibodies supports the concept of a difference in kind between what have been called SD (serologically defined) and LD (lymphocyte defined)¹⁴ gene products of the MHC.

In summary, we propose that immunological enhancement of transplants is mediated by MHC associated Ir antigen-antibody complexes, which implies that sensitisation to transplants is not due to differences between "transplantation antigens" (as recognised hitherto). On the contrary the differences we invoke are those between recognition molecules of passenger and/or resident lymphocytes in the transplant. Differences between lymphocytes measurable by mixed lymphocyte reactions are responsible for initiating the afferent arc in cell mediated lymphocytotoxicity; transplantation antigens (old style) are involved only in the effector arc¹⁵. These *in vitro* studies are lymphocyte/lymphocyte interactions and we cannot comment from our present *in vivo* results on whether the rejection process itself, as opposed to sensitisation for rejection, involves the transplantation antigens whose serological markers permit tissue typing.

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Physiology of Grain Filling in Barley

Walpole and Morgan¹ have suggested that differences in absolute growth rates between grains at different spikelet nodes in barley result from differences in quantity of assimilate supplied by each subtending awn, and that this quantity varies in proportion to awn size. They assume implicitly that awns affect grain growth rates by functioning solely as additional assimilate sources. If this is so, then differences in awn size between different nodal positions could determine these growth rate differences only if the assimilate requirements for grain growth were in excess of the total potential pool available for grain growth. Results from other work^{2,3} suggest that in barley (as in wheat^{4,5}) the converse situation may occur. From studies of temperature effects on the relationship between grain growth and leaf area duration^{6,7} and on CO₂ exchange rates⁸ in barley, it is a reasonable supposition that under the 'cool greenhouse' conditions used by Walpole and Morgan (with presumably a high light intensity and adequate water supply) the potential assimilate supply may have exceeded the grain growth demand in their plants. Support for this hypothesis is provided by their data as these show no evidence of any competition for assimilate between grains such as might be expected if demand had exceeded supply. If grain growth rates were not limited by assimilate supply, then either awns did not affect them or they did so primarily by affecting potential growth rates independently of assimilate supply, that is, by influencing the 'sink demand' (or 'sink capacity') of each grain.

The determinants of 'sink demand' of individual grains have not yet been satisfactorily resolved. Various suggestions have implicated the number of sites of starch synthesis and storage in each grain (as a function of endosperm cell number^{9,10} or cell size¹¹) and hormone(s) produced or accumulated within the grains which may directly promote or regulate the accumulation of organic and inorganic substances^{9,12-15} and/or influence the duration of synthetic activity in them¹²⁻¹⁴. It is possible too that endosperm size, and therefore the potential number of starch synthesis and storage sites, may be determined partly by the cytokinin content of grains over the period of cell division in the endosperm¹⁵.

Michael *et al.*¹²⁻¹⁴ have provided evidence that awns may increase the cytokinin content of grains in barley by increasing the transport of cytokinin from sites of synthesis (probably in the roots) to the grains, as a consequence of increased ear transpiration. Moreover, awn removal reduced grain size at a low air humidity, but not at a high humidity when transpiration was much lower¹²⁻¹³. Spraying cytokinin on the ears also increased grain size¹³. Differences in grain growth rates and final grain size were associated with differences in cytokinin activity in the grains¹⁴. This was so whether size differences were genetic in origin or due to treatments such as awn removal or water saturation of the soil. The differences in cytokinin content were

apparent before the differences in grain size. There is evidence too that awns may increase the transport of nitrogenous substances into the grains¹⁶.

None of this work provides substantive evidence that awns do affect grain growth rates by affecting 'sink demand', but it indicates in principle that this might occur. For example, during the period of endosperm cell division it is possible that a limiting availability of cytokinin and organic nitrogen compounds (rather than carbohydrates) in the grains frequently may limit volume growth of the endosperm and therefore subsequent growth rate potential during the starch storage phase. Awns of differing lengths, by affecting the quantities of these substances transported into the developing grains, might effectively vary endosperm size so that subsequent growth rates and final starch contents per grain are varied.

It seems likely that assimilate produced in the spike is used preferentially over that from the shoot for grain growth in barley, so that shoot assimilate satisfies demand in excess of spike supply^{2,17}. As each spikelet seems to supply assimilate only to its own grain^{1,2}, and as demand for shoot assimilate seems to be similar at each spikelet node¹, the implication is that if the 'sink demand' of individual grains is increased by increased size of subtending awns, then the additional demand can be satisfied by an increase in assimilate supply from within the spikelet. This might occur in one or more of several possible ways. The increased supply could derive from the longer awn itself. There might also be an increase in photosynthesis rate in the proximal source, that is the whole spikelet, in response to the increased assimilate demand—in the same way that flag leaf photosynthesis rate in wheat has been shown to vary with assimilate demand on the leaf¹⁸. There is evidence, too, that an enhanced availability of cytokinin in photosynthetic tissue may increase the activity of carboxylating enzymes^{19,20}. Photosynthesis rate in the whole spikelet thus might increase with increasing awn size as a result of enhancement by the awns of cytokinin availability in the spikelet tissue¹². Where the potential supply of assimilate already exceeded grain filling requirements, however, any awn effects on supply from the proximal source would be of secondary importance as any additional assimilate requirement deriving from awn effects on 'sink demand' presumably could be satisfied by increased export from the shoot.

Whether it is more usual for awns of varying size to affect the rate (and/or duration) of grain growth primarily by varying the supply of assimilate to the grains or by varying their 'sink demand' is a question which cannot be resolved until more critical work is carried out. For example, by using DCMU as a photosynthesis inhibitor, awn effects on grain growth could be determined in the presence and absence of awn photosynthesis.

On the basis of currently available information it is probably best to assume provisionally that awns play a multiple role in the determination of grain growth rates and final grain size, with the dominant function depending on genotype and environment. Until more is known of the physiology of the awn-grain relationship it may be premature to specify awn characteristics as selection criteria in breeding programmes for increased yields in barley¹.

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Protein Pellicle of Stigmatic Papillae as a Probable Recognition Site in Incompatibility Reactions

ANGIOSPERM pollen grains carry extracellular proteins in two sites: in the inner wall layer, the cellulosic intine, and in cavities in the outer exine, the sculptured part of the wall composed of sporopollenin¹⁻³. The intine proteins, which include several acid hydrolases, are synthesised by the spore and inserted during growth, they are therefore gametophytic in origin. The exine proteins, in contrast, are produced in the tapetum, the nurse tissue of the anther, and injected into the exine cavities during the maturation of the pollen grains; they are thus sporophytic. The enzymic wall proteins seem to be concerned with germination, penetration of the stigma surface and the early growth of the pollen tube. Other fractions are involved in the recognition responses which control breeding behaviour, playing a part in both interspecific⁴ and intraspecific^{5,6} incompatibility systems.

Protein fractions separated from the exines of species of Cruciferae and Compositae can induce characteristic responses in stigma hair cells. Most notably, extracts from incompatible pollen will elicit a rejection reaction in stigma papillae, a reaction which leads ultimately to the deposition of callose in cells in the immediate vicinity of areas of contact⁵. 'Recognition' in incompatibility systems of this kind presumably depends upon interactions between specific pollen proteins and complementary fractions associated with the stigma or style^{7,8}. When the rejection of unacceptable pollen occurs at the stigma surface, as is often found in cases of interspecific incompatibility and in sporophytically controlled intraspecific incompatibility systems, the receptor sites must be in, or upon, the stigma hair cells. In seeking to locate these sites, we have found that the surfaces of the stigma papillae bear an external protein coating overlying the cutinised outer layer of the wall. This coating, which we shall refer to here as the pellicle, has certain properties which lead us to believe that it is functionally important in the capture and hydration of pollen grains. The layer could also be the site of the recognition reactions involved in incompatibility responses.

The principal observations were made on stigmas of *Silene vulgaris* (Caryophyllaceae) and *Brassica oleracea* and *Raphanus sativus* (Cruciferae). In a wider survey, the presence of the pellicle has been confirmed in some eighty other angiosperm families, indicating that it may well be universal in the group. The existence of the layer was first

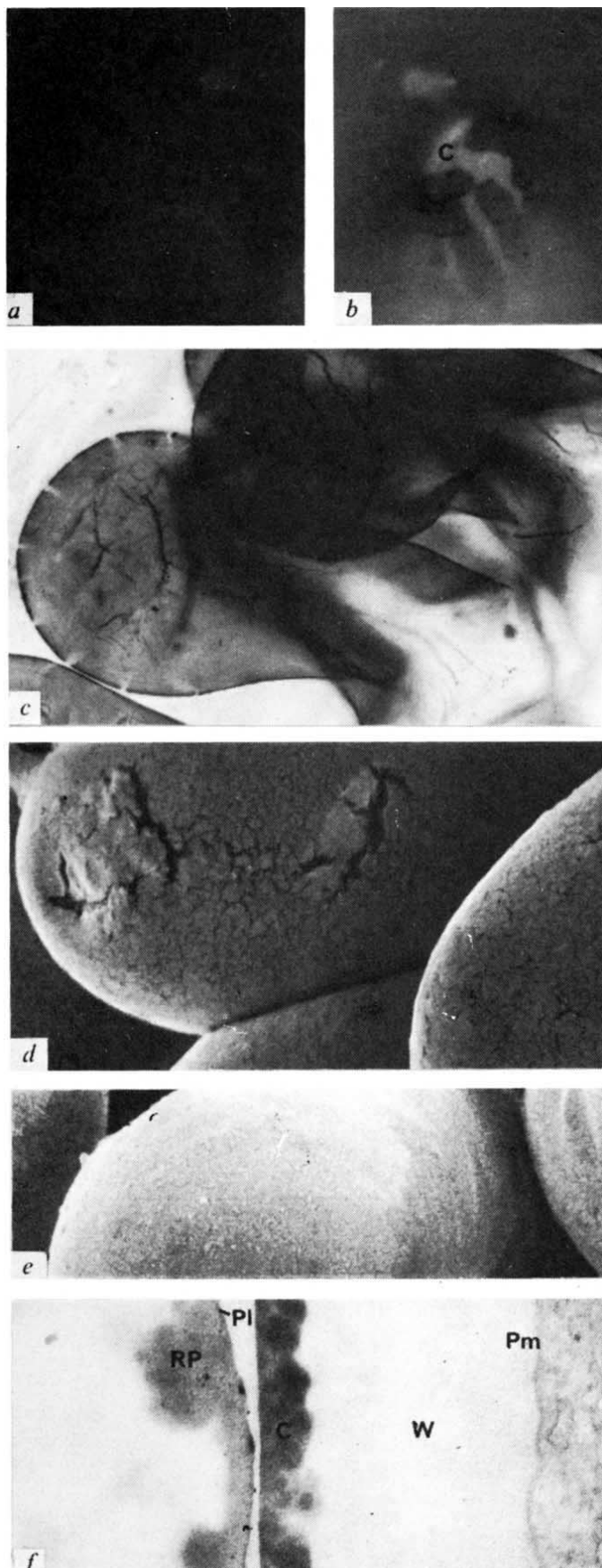
noted when, using fluorescein diacetate as a substrate⁹, it was found that esterase activity is associated with a layer lying outside of the cuticularised wall of the stigma papilla. More precise histochemical localisation of non-specific esterase¹⁰ confirmed this distribution. The pellicle was revealed directly with the fluorescent protein stain, 1-anilino-8-naphthylsulphonic acid (1-ANS), used as described elsewhere⁵. Digestion experiments were carried out with Pronase (BDH, 1 mg ml⁻¹ in 0.02 M Tris buffer, pH 7.2, 1 h at 37° C) and lipase (Sigma Type VII, 2 mg ml⁻¹ in 0.1 M Tris buffer, pH 7.5, 1 h at 37° C). The benzpyrene technique¹¹ was used for the localisation of the cuticle underlying the pellicle. For transmission electron microscopy, whole stigmas were prefixed at room temperature for up to 24 h in 1.5% glutaraldehyde in 0.05 M phosphate buffer at pH 7.0 containing 12% sucrose, and then transferred to 1% osmic acid in the same buffer for 1-2 h at 3-5° C. The fixed material was embedded in Araldite, and thin sections prepared by standard methods. For scanning electron microscopy fresh or pre-treated stigmas were freeze-dried and coated with gold-palladium alloy.

The cleavage of fluorescein diacetate by non-specific esterases releases soluble fluorescein which will normally diffuse from the enzyme site. Some local binding, however, takes place in the pellicle or below it, so the superficial enzyme activity can be detected in a few minutes using this substrate. The esterase activity of the pellicle is, however, best demonstrated using α -naphthylacetate as a substrate in a simultaneous coupling reaction with either Fast Blue B salt¹⁰ or hexazotised pararosanilin¹², where the reaction products are not diffusible. The distribution is the same with the two coupling agents, activity being present over the whole outer surface of the papillae, grading away towards the base (Fig. 1c). This distribution coincides precisely with that of a protein layer observable by 1-ANS staining (Fig. 1a). So far, no other hydrolytic enzymes have been detected in the pellicle, and it is noteworthy that there is no acid phosphatase activity, although this is always associated with the pollen intine².

The protein pellicle ensheaths the cuticle, but without any close attachment. It readily forms cracks and fissures on the older stigma (Fig. 1c and d), and then the cuticle can be viewed through it after benzpyrene staining (Fig. 1b). As comparison of the two scanning electron micrographs (Fig. 1d and e) shows, the layer is dispersed by Pronase digestion. After its removal the underlying cuticle remains intact, giving the usual cytochemical reactions. So far as can be detected by optical microscopy, lipase treatment does not modify the pellicle.

The stratification of the wall at the tip of a stigma papilla and the location of the pellicle are seen in the electron micrograph of Fig. 1f. The plasmalemma is often irregular in this part of the cell. It may adjoin the pecto-cellulosic wall directly, but there are often intervening inclusions with an electron density after standard stain suggesting a proteinaceous nature. The pecto-cellulosic layer is bounded by a cuticle, but this does not form a continuous layer. There are no regular channels of the nature of ectodesmata, but the cutin itself is disposed in bars and rods, with many discontinuities offering communication from the outer face to the underlying pecto-cellulosic wall.

A protein pellicle of the kind described here seems not previously to have been reported from the external surfaces of higher plant cells. Cutinised epidermes frequently bear waxy overlays which enhance their water repellency, but not protein layers which from their hydrophilic nature might be expected to increase wettability. Clearly, however, the presence of a hydrophilic pellicle does help to explain one aspect of the function of stigmatic papillae. The stigmas in many flowering plant families are superficially dry, like those of Caryophyllaceae and Cruciferae, but of course they provide efficient attachment surfaces for pollen; and it is



well known that after capture pollen grains become hydrated very rapidly (in *Silene*, for example, volume increases by some 50% in the first 10 min after contact). Three forces are likely to be involved in initiating and maintaining this water movement: capillarity at the surface of contact of pollen wall and pellicle; the hydration of colloids, particularly at the germination apertures of the pollen grain; and, ultimately, osmotic forces as the protoplast of the vegetative cell of the pollen grain begins to vacuolate. The structure of the wall of the stigma papilla suggests that water could be withdrawn through the cuticle underlying the pellicle by way of the discontinuities in this layer, the flow being from the pecto-cellulosic inner wall, which in turn would withdraw water from the turgid protoplast of the stigma hair cell. It may be noted that the mechanism envisaged would have a self-regulatory element. While the hair cells remained turgid, the wall would be distended, the interruptions in the cutinised layer would be gaping, and so the protein pellicle would remain hydrated and presumably adhesive. With incipient drying, such as might be expected in an open flower in the Sun, the stigma hair cells would lose turgidity and shrink; and as the cuticle became less stretched the discontinuities would close, so retarding further water loss. The pellicle would then dry out, and presumably become less receptive. The process would be reversed once the water balance was restored.

Beyond the function of the pellicle in the capture and hydration of pollen, there is the possibility that it might be concerned in pollen-stigma interactions in another way, as the site of the recognition responses of incompatibility systems. It is known that the hydration of the pollen grains upon the stigma is accompanied by the emission of proteins and other mobile fractions carried in the exine and intine^{13,14}. The emitted enzymes are probably concerned with germination and the penetration of the stigma surface by the pollen tube¹⁵, and among the non-enzymic protein fractions are those involved in incompatibility reactions^{5,6}. Observations on *Silene* and various Cruciferae show that emitted pollen proteins bind to the pellicles of the stigma papillae very quickly after the initial contact, a strong indication that this is the primary recognition site. Significantly, the erosion of the cuticle of the papilla in anticipation of the entry of the pollen tube begins under the binding zones. This might suggest a cutinase 'activation' in the manner proposed by

Fig. 1 *a*, Unfixed stigma papillae of *Raphanus sativus* stained *in vivo* with 1-ANS; fluorescence micrograph. The protein pellicle is seen towards the tips of the stigma papillae, about $\times 750$; *b*, stigma papilla of *Silene vulgaris*, esterase reaction with α -naphthylacetate as substrate in a coupling reaction with Fast Blue B salt, stained also by the benzpyrene method for cutin. The pellicle is seen as a dark sheath investing the cuticle, but where it is torn away the underlying cutin shows the characteristic benzpyrene fluorescence, about $\times 1,000$; *c*, whole stigma papillae of *Silene vulgaris*, esterase reaction as in *b*. The pellicle shows intense activity especially towards the bulbous tips. The discontinuities seen in the profile here are due to cracks and tears, about $\times 1,850$; *d*, stigma papillae of *Brassica oleracea*. Scanning electron micrograph of freeze-dried, unfixed material. The pellicle is clearly seen, with numerous cracks and fissures, about $\times 3,250$; *e*, as *d*, but stigma surface exposed to Pronase digestion. The pellicle has been wholly removed, revealing the underlying cuticle, about $\times 3,250$; *f*, transmission electron micrograph of the wall of a stigma papilla of *Silene vulgaris* towards the tip. The fresh stigma was exposed to an esterase reaction with α -naphthylacetate as substrate in a coupling reaction with hexazotised pararosanilin. The stigma was then fixed in glutaraldehyde and osmicated as in the text. Thin section without post-staining. The pecto-cellulosic wall (W) adjoins the plasmalemma of the papilla (Pm) on the right, with the outer cuticle towards the left (C). The pellicle overlying the cuticle (Pl) is made conspicuous by the associated esterase reaction product (RP) rendered moderately electron opaque by osmication, about $\times 23,000$.

Linskens and Heinen¹⁶, and perhaps offer some clue as to the meaning of the cytochemically detectable 'esterase' activity of the pellicle itself.

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Mitosis in the Cryptophyceae

IN a recent issue of *Nature*¹ Oakley and Dodge are critical of my theory on the evolution of the algae². I believe that their criticisms are the result of some misconceptions and inaccuracies. In reference to my article² they state "the Cryptophyceae were thought to be ancestral and closely related to the Cyanophyceae". First, there must be some error in sentence structure, for to conclude that the eukaryotic Cryptophyceae are ancestors to the prokaryotic Cyanophyceae is a difficult assumption to make. Second, I never said that the Cyanophyceae were closely related to the Cryptophyceae but that the Cyanophyceae were closely related to the chloroplasts of the Cryptophyceae. Therefore one would not expect cell division in the Cyanophyceae to be similar to that in the Cryptophyceae as they infer.

Oakley and Dodge refer to McDonald's work on mitosis in the Rhodophyceae³ and state that "It (mitosis in the Cryptophyceae) is quite different from that in the Rhodophyceae" without expanding further. I believe that they have overstated their case, as the only differences in mitosis between the two classes are: (1) the presence of some heterochromatin referred to as a kinetochore in the Rhodophyceae with no such structure in the Cryptophyceae, and (2) the presence of basal bodies in the Cryptophyceae and their absence in the Rhodophyceae (which would be expected since the Rhodophyceae have no flagellated cells) although the polar ring³ in the latter may prove to be a derivative of a basal body. These differences between mitosis in the two classes are not as significant as Oakley and Dodge seem to believe and do not rule out an evolutionary link between the Cryptophyceae and Rhodophyceae.

Lastly, Oakley and Dodge infer that *Chroomonas* (a Cryptophyte with a chloroplast) is a primitive genus in my scheme on the phylogeny of the algae². This is not so as this type of organism is fairly advanced along the evolutionary pathway. If they were seeking a more primitive organism to investigate they should have chosen a Cryptophyte without chloroplasts or one with cyanelles (endosymbiotic Cyanophyceae).

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Drs Oakley and Dodge reply: In regard to Dr R. E. Lee's correspondence, our letter to *Nature*¹ was not intended to be particularly critical of Dr Lee's previous article on endosymbiosis and the evolution of the algae². In fact Dr Lee's theory was only mentioned very briefly. Nevertheless we should like to respond to his comments. Of course when we stated that "the Cryptophyceae were thought to be ancestral and closely related to the Cyanophyceae", there was no intentional implication that the Cryptophyceae were ancestral to the Cyanophyceae but rather that they were ancestral algae. Since Dr Lee's scheme regards them as ancestral to every group of algae other than the Cyanophyceae we feel that it is fair to regard them as ancestral. Although Dr Lee does not state explicitly that the Cryptophyceae are closely related to the Cyanophyceae, his chart (Fig. 1) giving phylogenetic relationships places two groups of cryptophytes, the colourless cryptophytes and those with cyanelles, closer to the Cyanophyceae than any other groups of algae. We do not feel, therefore, that our statement was unfair.

This point, however, is of little importance. A much more important point, and the point we were making, is that Dr Lee's scheme implies that the Pyrrophyta with very little histone³, extremely unusual base pair composition⁴, and an apparently primitive mitotic apparatus⁵ has evolved from the Cryptophyceae which show no such apparent primitiveness or uniqueness. Similarly, the mitotic apparatus of the Euglenophyceae seems to be much more primitive than the cryptophytes from which they have evolved according to Dr Lee's scheme.

In fairness, Dr Lee does not feel that the cryptophytes with chloroplasts are ancestral to the Pyrrophyta. He feels that the colourless cryptophytes and those with cyanelles are more primitive than those with chloroplasts. With regard to the colourless cryptophytes, our results were so similar to the light

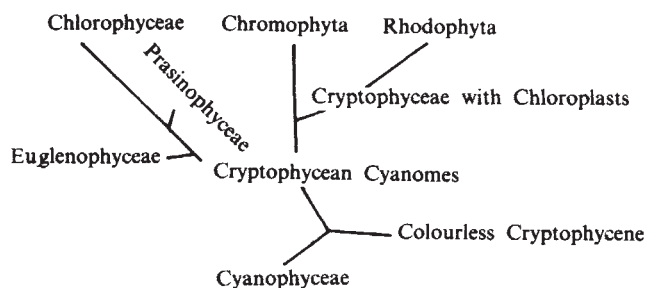


Fig. 1 Fig. 2 from ref. 2. The origin of plastids from a Cyanophyceae alga involved in an endosymbiosis with a colourless Cryptophyte. The remainder of the eukaryotic algae then evolved from this Cryptophyceae cyanome (endosymbiotic Cyanophyceae alga plus the host cell).

microscopic studies on *Chilomonas paramecium*^{6,7} that there was no reason to believe that there might be any difference between the coloured and colourless cryptophytes. In addition, there is recent evidence that the colourless cryptophytes possess degenerate chloroplasts⁸ and may not be ancestral to the cryptophytes with chloroplasts at all; the reverse being the case.

In regard to *Cyanophora paradoxa* which Dr Lee regards as a cryptophyte with a cyanelle, though this organism certainly seems to possess a cyanelle, there is some question as to its being a cryptophyte. Moreover, mitosis has been studied in this organism⁹ and it shows no primitive characteristics.

Finally, Dr Lee does not feel that the presence of kinetochores and the lack of basal bodies in the Rhodophycean alga previously studied¹⁰ are particularly significant, but to us these seem to be quite significant differences. The presence of the unique polar ring and the near complete retention of the nuclear envelope during mitosis in the Rhodophyceae also constitute striking differences. These differences suggest that the mitotic apparatus of the Cryptophyceae is more nearly like that of several other groups of algae than that of the Rhodophyta.

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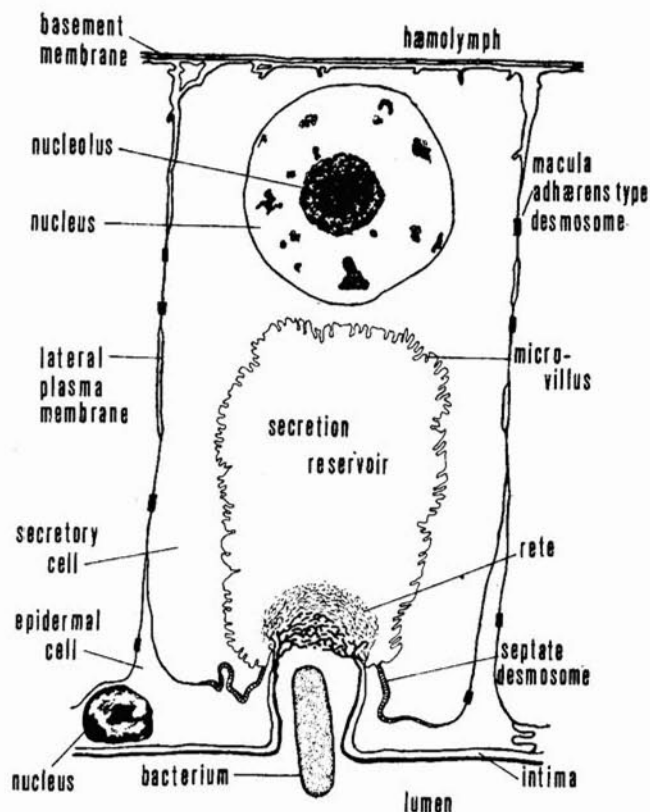


FIG. 1 Schematic representation of the structural organization of the milk gland tubules in *Glossina morsitans*.

Ultrastructural details were determined by fixation of the gland-fat body complex for 6 h in cold 5% glutaraldehyde buffered at pH 7.4 with 0.05 M sodium cacodylate buffer and post-fixation in buffered 1% OsO₄ for 1½ h. Sections from Epon-Araldite embedded materials were cut on a LKB Ultratome III using glass knives, mounted on unsupported copper grids, contrasted with saturated uranyl acetate in 50% ethanol followed by lead citrate, and examined with a Zeiss EM 9A electron microscope.

The walls of the gland tubules consist of two layers of cells: a single layer of squamous epidermal cells of ectodermal origin which lines the lumen, and a layer of secretory cells resting on a thin basement lamina. The epidermal layer is continuous with the non-glandular epithelium of the two efferent ducts and has a smooth unlaminated cuticular lining. The efferent ducts themselves have a helically thickened cuticle.

The accessory substances produced by the secretory cells are released by merocrine secretion into an extracellular storage space formed by invagination of the plasma membrane along the apical surface of the gland cell. One such storage reservoir is associated with each gland cell (Figs 1, 2). The stored secretion is channelled into the lumen through a cup-shaped invagination of the intima which projects into the reservoir. At the point of contact with the reservoir the intima is composed of 50–70 nm thick anastomosing strands forming a rete of 2.0–2.5 µm diameter. Underneath this coarse extracellular porous structure the cuticle is further ramified into a mass of extremely fine threads of about 3–6 nm diameter which has a wool-like appearance in the electron micrographs (Fig. 3). This layer is about 1.1 µm thick in the centre. There is no evidence that structural changes or deformations of the ductule occur during cycles of secretory activity. The cuticular wool however has a slightly blurred appearance in the electron micrographs when it becomes saturated with the

Secretory discharge and microflora of milk gland in tsetse flies

THE uterine or milk glands in tsetse flies (*Glossina* spp.) are modified female accessory reproductive glands which elaborate and release a nutritive liquid of proteinaceous and lipid nature for the maturing intrauterine larva^{1–3}. The multi-branched tubules of the gland converge into a pair of efferent ducts which fuse inside the oviductal shelf and open into the lumen of the uterus just posterior to the opening of the oviduct^{4,5}. Cytological details of the milk gland and their modulations in relation to the state of pregnancy of the female have been described⁶ (W-C. M., D. L. D., D. S. Smith and U. Jarlfors, in preparation). Earlier work has suggested that milk is released directly into the lumen of the gland by apocrine secretion⁴. Our observations on the structure of the milk gland do not support such a mechanism, but rather, a novel type of exocrine discharge in which secretion is stored in an extracellular reservoir and released into the lumen through a dense cuticular network. At the points of milk release the lumen is frequently inhabited by bacteria which have not previously been described in milk glands. Our examination is based on milk glands from *G. morsitans morsitans* Westwood, but a comparative study using *G. austeni* Newstead and *G. longipalpis pallidipes* Austen has shown no essential differences among the three species.

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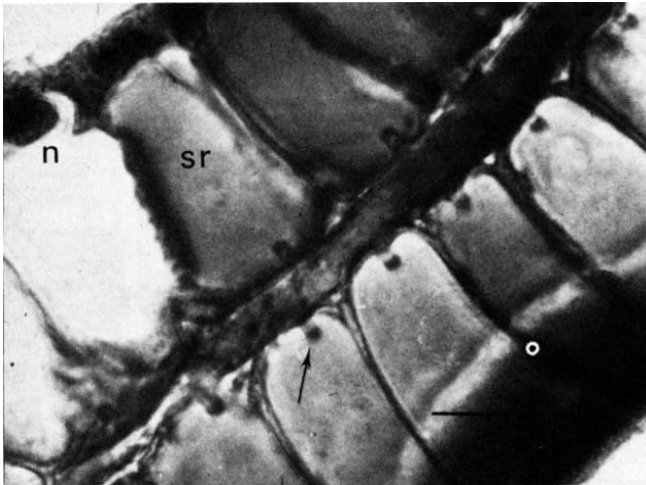


FIG. 2 Longitudinal section through a gland tubule at 6 d after ovulation showing the fully loaded secretory reservoirs (sr), a nucleus (n) of a gland cell, and the ductules projecting from the intima into the reservoirs (arrow). Paraplast embedding, Ehrlich's haematoxylin-eosin, phase contrast. (Calibration 0.1 mm).

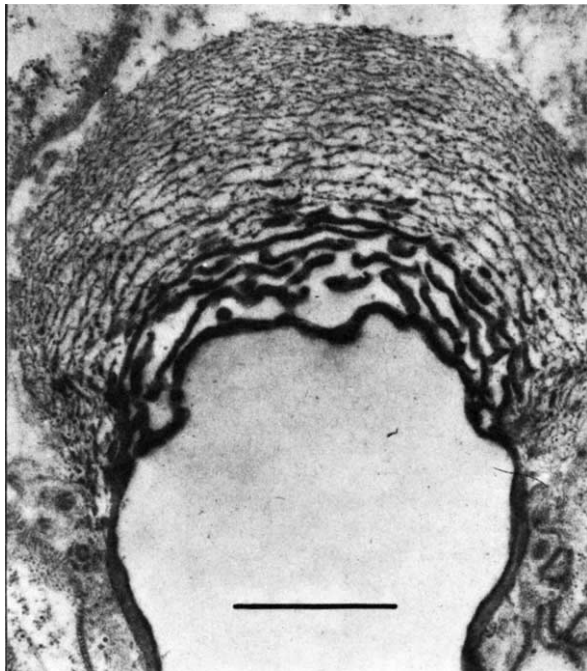


FIG. 3 Electron micrograph of a pore opening in the cuticular intima showing the rete with wool-like cuticular mass. The picture was taken from a secretory inactive gland. (Calibration 0.1 μm).

flocculent or finely granular textured proteinaceous milk secretion during secretory active phases of the gland. The remarkable construction of the pore opening could be functional in providing a high tensile strength, likely to be required to resist stress across the pore opening during cyclic increase in hydrostatic pressure in the reservoirs. Protection against possible rupture of the pore openings during periods of maximal reservoir expansion is further obtained by tight linkage of the plasma membranes of the epidermal cells and the apical surface of the secretory cells by septate desmosomes in the region of the rete. Ruptures have not been observed by us, but an accidental occurrence may account for the apocrine secretion described by Hoffmann⁴.

Myofibrils are lacking around the gland tubules, but the 300–350 μm long paired efferent ducts are surrounded by several layers of muscle cells which extend over a length of 200 μm . As illustrated in Fig. 4 the thickness of the muscle layer is at a maximum distally. From a maximum thickness of 20 μm the muscularis decreases in size and disappears completely before reaching the flattened region of the ducts near the oviductal shelf. Circumferential contraction of the muscles therefore produces the greatest force per unit surface area in the distal portion of ducts. Since the glandular part is a closed system the milk can only flow towards the uterus. The high resistance in the narrow and flattened duct region increases the velocity of flow during passage causing the milk to be ejected into the common milk gland duct in the oviductal shelf. At the same time the flattened duct region, also observed in *G. austem*⁷ could function as a valve promoting unidirectional flow in the efferent ducts during alternating muscular con-

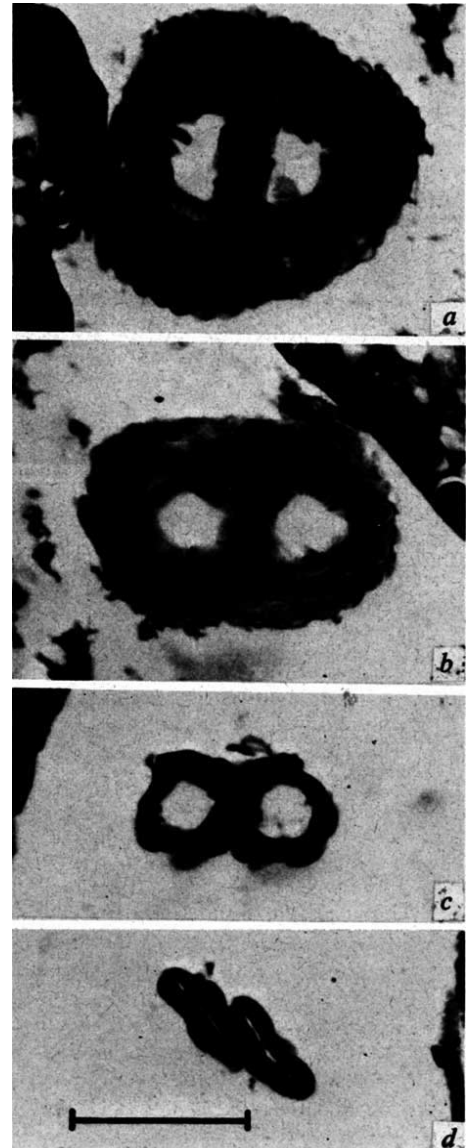


FIG. 4 Cross sections of the efferent ducts taken at intervals of about 30 μm , arranged in order from close to the site of bifurcation into the glandular part a, to near the oviductal shelf, d. Paraplast embedding Ehrlich's haematoxylin-eosin. (Calibration 50 μm).

traction and relaxation. Relaxation of the efferent duct muscles after milk ejection would result in a lowering of hydrostatic pressure within the lumen of the gland tubules. The difference in pressure could regulate the outflow of the secretion from the secretory reservoirs into the lumen. The increase of positive hydrostatic pressure within the secretory reservoirs during accumulation of secretion would facilitate outflow, but in itself cannot be responsible for flow regulation. The ejected milk accumulates in the space around the anterior region of the larva, a region which is sealed off from the rest of the uterus by uterine ridges⁵. Suctional forces exerted by the feeding larva could contribute to the amount of milk flow during ejection and may be a prerequisite for its repeated occurrence.

The cuticular rete associated with the pore openings provides an effective barrier against possible invasion of the reservoirs or cells by microorganisms. In fact, the cup-shaped invaginations of the intima are commonly occupied by a rod-shaped, fimbriated type of bacteria (Fig. 5). Whereas these bacteria are numerous in the lumen of the milk gland tubules, they rarely are found in the lumen of the efferent ducts. The bacteria vary in length from 3.4 to 4.5 μm and are 1.1 μm in width. The triple-layered cell wall (total thickness of about 13 nm) consists of two outer dense layers separated by a much less dense layer. A clear intermediate zone of 12–15 nm separates the cell wall from the poorly contrasted plasma membrane (Fig. 6). The cell wall structure coincides with that of Gram-negative bacteria⁸, although in the latter the general thickness is about 8 nm. The fimbriae, scattered over the whole cell surface, are 5–7 nm in diameter and about 2 μm long. The bacterial cytoplasm contains scattered electron-dense agglomerations, diffuse filamentous structures, and concentrations of ribosome-like granules. Reproduction is by binary fission following an elongation to about 6.5 μm ; a cross-wall is not formed. These properties together with the possession of fimbriae and the absence of mesosomes are commonly shared by Gram-negative, but generally absent in Gram-positive bacteria⁹.

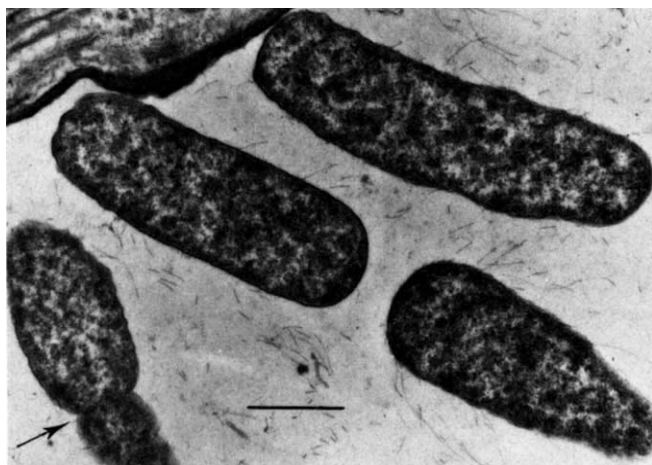


FIG. 5 Electron micrograph of fimbriated bacteria in the lumen of the milk gland. The arrow points to one bacterium during fission. (Calibration 0.1 μm).

The bacteria were observed in the milk gland lumen of all flies ($N = 64$) examined over a period of 1 yr. Since the rabbit population on which the flies were maintained was replaced frequently it seems unlikely that the otherwise perfectly healthy flies could have been infected with a

bacteraemia from the rabbits. Moreover, in case of such an infection it would be difficult to imagine how the bacteria could penetrate the gut wall and gland epithelium to reach the milk gland lumen which is lined with cuticle. The bacteria were never observed inside the gland epithelium,

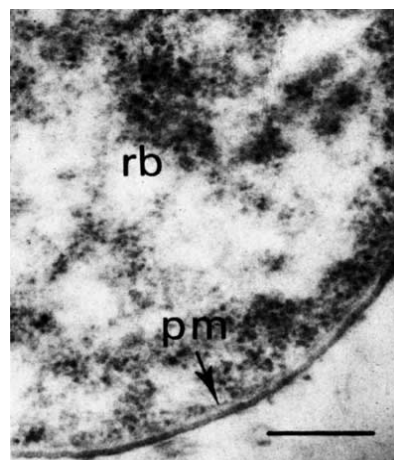


FIG. 6 Part of a symbiotic bacterium with plasma membrane (pm), cell wall, and ribosome-like grana (rb). (Calibration 0.3 μm).

nor in the haemolymph or fat body surrounding the gland tubules. Therefore, we interpret the bacteria in the milk gland to be symbiotic microorganisms. It is of interest to note that the symbiotic intracellular microorganisms (bacteroids) occurring in the giant-cell zone of the anterior midgut of *Glossina* have been suggested to be hereditarily transmitted by the maternal secretion from the milk gland^{10,11}. The ultrastructure of the gut bacteroids¹² deviates from the gland bacteria in showing a thinner cell wall (though of similar structure), and the absence of fimbriae. But on the basis of these morphological differences alone it is not possible to reject the hypothesis that the fimbriated bacteria represent the free-living form of the intestinal bacteroids. In *Salmonellas*, fimbriae have been suggested as functional structures for cellular invasion.¹³

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Vaccination of Rhesus monkeys against Malaria by use of sucrose density gradient fractions of *Plasmodium knowlesi* antigens

WE wish to report the results of immunity induction trials in the *Plasmodium knowlesi*-Rhesus monkey system. Thirteen juvenile (1.5-2.0 kg) Rhesus monkeys (*Macaca mulatta*) were vaccinated with various non-viable fractionation products derived from the plasmodial parasite, *P. knowlesi*. Samples were prepared from parasitised erythrocytes by differential French Pressure Cell disintegration and centrifugation, Bio-Gel column chromatographic separation, and linear sucrose density gradient fractionation. Two injections of the respective antigen preparations, emulsified in Freund's adjuvant, were given at 4-week intervals, followed 4 weeks later by blood-stage challenge with the homologous *P. knowlesi* strain. The resulting data reflect a significant difference in peak parasitaemias of the vaccinated monkeys as compared to control monkeys.

Outlined in Fig. 1 and its legend are the preparatory procedures used in obtaining the various parasite fractions which were subsequently tested for antigenic potential. Experimental treatments and results are described in Table 1 and Fig. 2.

The first injection of the immunisation schedule used Freund's Complete Adjuvant (Difco, Detroit) combined with an equal volume of antigen. The second injection, 1 month later, consisted of an equal volume of antigen emulsified in Freund's Incomplete Adjuvant. Groups 1 and 4 were injected each time with 1.0 ml of antigen preparation (average A_{280}

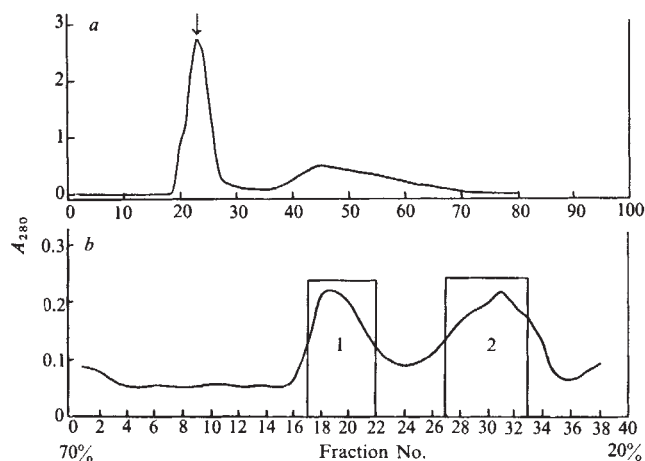


FIG. 1 The first stage of antigen preparation followed the protocol detailed by Schenkel *et al.*^{1,5} with the exception that final French Pressure Cell (FPC) effluent was subjected to molecular sieve column chromatography on Bio-Gel A1.5 (Bio-Rad, Richmond, California), rather than on Sephadex G-200. Subsequent fractionation as described in this report focused on the void volume eluate, (arrow, Fig. 1a), shown in earlier studies to contain the immunising material, and to be free of detectable host cell contamination¹⁻⁵. Fractionation of the Bio-Gel void volume eluate was accomplished by linear sucrose density gradient centrifugation⁶. The gradients, with terminal concentrations of 20-70%, were prepared by means of a gravity flow apparatus at 4° C. Each cellulose tube contained a total of 29.0 ml. Centrifugation (at 25,000 r.p.m. for 240 min at 4° C) was carried out in the Beckman Model L ultracentrifuge, equipped with a SW 25.1 head (Beckman, Spinco Division, Palo Alto, California). Samples (0.7 ml) were collected by means of a fractionator using needle puncture of the gradient tubes. A typical profile is seen in Fig. 1b. Vaccination preparations consisted of respectively pooled peaks 1 and 2. Sample pools were dialysed overnight against PBS (2-3 changes) to remove the sucrose.

TABLE 1 Treatment, survival, and peak parasitemias of vaccinated and control monkey challenged with *Plasmodium knowlesi*.

Treatment	Group No.	Monkey No.	Monkey surviving challenge = +	Peak parasitemia after initial challenge
Bio-Gel void volume eluate preparation in Freund's adjuvant	1	1486 1481 1493	+ + +	<0.5 7.5 2.5
Peak 1 sucrose density gradient fraction in Freund's adjuvant	2	1484 1489 1491	- - +	0.0 0.5 2.5
Peak 2 sucrose density gradient fraction in Freund's adjuvant	3	1483 1492 1497	- + +	6.0 <0.5 <0.5
Bio-Gel void volume eluate-no adjuvant*	4	1476 1477	- -	50.0 58.0
Freund's adjuvant-no antigen	5	1479 1480	- -	24.0 48.0

* In earlier published data¹ the void volume eluate without adjuvant had been shown to be non-protective, thus, this group constituted an additional control.

2.0). Groups 2 and 3 received an average of 2.0 ml of the respective antigen preparation (average A_{280} 1.0) at each injection. Concentrations of the respective colloidal antigen preparations used for immunisation were approximated by absorbancy at A_{280} . More exacting measurements were not deemed necessary in view of the relative impurity of the preparations and the linearity of absorbancy to protein concentration. Group 5 received 1.0 ml of Freund's adjuvant at each injection. Injections were given intramuscularly (IM) in opposite thighs.

The challenge inoculum consisted of approximately 2×10^7 *P. knowlesi*-infected red blood cells, given intravenously 1 month after the second antigen injection. The progress of the infection was monitored by percentage parasitaemias, red blood cell counts, and microhaematocrits. (Due to the fulminant nature of the infection, determination of exact parasitemias at death was difficult to obtain. For this reason, the observed parasitemias of unprotected animals may have been, in many cases, artificially low.)

The significance of differences in mortality rates was calculated by Fisher direct *P* significance test.⁷

All surviving monkeys were rechallenged with 1×10^8 parasitised RBC of the homologous *P. knowlesi* strain 3 months after the initial challenge. At that time the red blood cell counts and microhaematocrit values of these animals had returned to prechallenge levels, and no parasites were detectable in peripheral blood smears.

The monitored responses to the initial challenge can be generally categorised into three classes: 6/9 vaccinated animals (Groups 1, 2 and 3) which demonstrated parasitaemias of 0-7.5% and survived; 3/9 vaccinated animals (Groups 1, 2 and 3) which demonstrated similar parasitaemias, but did not survive; and, 4/4 control animals (Groups 4 and 5) which demonstrated extremely high parasitaemias of up to nearly 60% and died.

In Group 1, all three animals survived challenge with the average maximum parasitaemia being 4%. In Group 2, two of three animals died after challenge, although the parasitaemias of all animals in this group were extremely low (average maximum parasitaemia, 1.3%). At necropsy one of the two non-surviving monkeys (1489) showed evidence of a respiratory infection. Two of three monkeys in Group 3 survived challenge. The parasitaemias of all animals were very low and that of the survivors did not exceed 0.5%.

A wide range of indirect evidence⁸ suggests that Peak 1 contained primarily the malarial pigment, haemozoin, together with protective antigenic material. Peak 2 seemed to be essentially free of iron, as assessed by atomic absorption

spectrophotometry, and contained protective antigenic material. Thus, it had been anticipated that both sucrose gradient peaks would confer protection against a challenge of *P. knowlesi*.

All animals in Groups 4 and 5 died of a rapidly fulminating infection, reflecting the normal course of the disease in non-protected Rhesus monkeys. The parasitaemias and RBC counts of these monkeys were indistinguishable from the numerous non-injected controls used in previous experiments. The lack of protection of animals in Group 4, which received Bio-Gel void volume eluate without adjuvant, corroborated our earlier experiments and those of other investigators^{1,9,10}, and emphasised the importance of combining the antigen with an effective adjuvant. Group 5 represents a control to detect non-specific resistance induced by Freund's adjuvant alone. No such resistance was observed.

The peak parasitaemias of the nine animals in experimental Groups 1, 2 and 3 were similar, whether or not the animals survived, and were significantly lower than those of the animals in the control Groups 4 and 5 (Table 1). Figure 2 indicates that the course of the anaemia was similar in all animals.

Upon rechallenge none of the surviving monkeys succumbed to the infection and peak parasitaemias were not in excess of 1%.

These results confirm and extend the earlier findings of Schenkel *et al.*^{1,5} concerning the feasibility of vaccinating sub-human primates. However, one of the more striking observations concerns the drop in red blood cell profiles following challenge. After a slight initial rise in both RBCs and microhaematocrits, the blood indices dropped dramatically, even in protected groups where parasitaemias remained <5% or were entirely undetected. A similar but smaller decline was observed upon rechallenge of surviving animals.

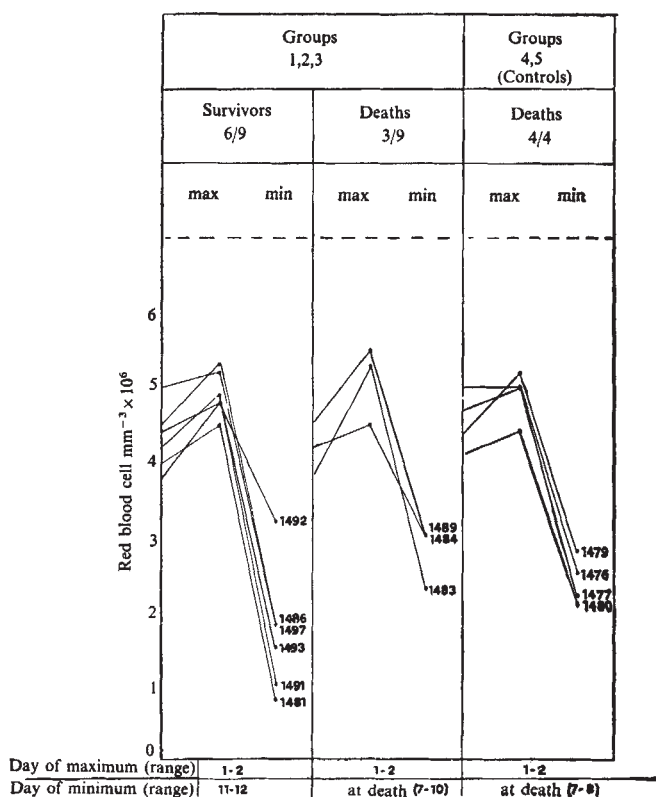


FIG. 2 Maximum and minimum RBC counts following initial challenge. Animals are divided into the three defined classes (vaccinated-surviving; vaccinated-fatality; control).

The aetiology of the anaemia could be postulated as due to some complication of the vaccination process and/or the natural course of the infection, as erythrocyte destruction greater than that predicted from the percentage of cells infected has been well documented¹¹⁻¹⁸. Also, certain factors of the present experimental design may be relevant. Unlike previous trials, in this experiment juvenile Rhesus monkeys (1.5 kg weight), rather than adult animals (6 to 7 kg), were used, thus more closely simulating the immune status of children, and the challenge inocula were extremely status of (Analysis of minimum infective dosage in the *P. knowlesi*-Rhesus monkey system has demonstrated that the number of parasitised erythrocytes sufficient to cause death approaches five: L. Schmidt, personal communication.) The contributions of each of these parameters, or combination of parameters, to the observed haemolysis is unknown.

In this study, mortality figures (antigen in Freund's adjuvant versus control groups) become significant to less than 1% only when the results of the preceding vaccination series^{1,5} are included. Summation of the data produces a total series of twenty five monkeys. Eleven of seventeen vaccinated animals have survived challenge, while none of the eight control animals have survived ($P < 1\%$).

In summary, we have demonstrated that a significant decrease in parasitaemia and an increase in survival of Rhesus monkeys infected with *P. knowlesi* can be induced by the administration of non-viable sucrose density gradient fractions, in Freund's adjuvant, of the void volume preparation used successfully in earlier studies^{1,5}.

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Binding of thrombin to human platelets

MUCH work has been carried out aimed at elucidating the mode of action of thrombin on platelets¹⁻⁵. These studies were based on the assumption that hydrolysis of a protein by thrombin constitutes the primary event culminating in aggregation of platelets. Results of recent studies suggest that the primary step in this process might be binding of thrombin to a receptor site, triggering viscous metamorphosis of the platelets^{6,7}. The processes of binding and proteolysis need not be mutually exclusive, however, binding may be followed by proteolysis. Here I present direct evidence of thrombin binding by human platelets and discuss some of its implications.

Human blood from volunteer donors was collected in acid-citrate-dextrose in plastic bags and the red cells were removed by differential centrifugation. The platelet-rich plasma (PRP) was recovered in a plastic bag in less than 2 h after collection. Siliconised glass or plastic materials were used throughout this study. The platelets from PRP were isolated and washed by the density gradient method using sodium metrizoate (Nyegaard Co., Oslo) as a cushion⁸. The final volume of the washed platelets in modified Tyrode's solution was adjusted so as to restore the platelet concentration to approximately that of the original PRP.

Bovine thrombin (Parke Davis, Detroit, Michigan) was purified by ion exchange chromatography on SP-Sephadex C-50 (ref. 9). The specific activity varied between 1,200 to 1,700 units mg^{-1} of protein. Experiments using US Standard human thrombin (NIH, lot H-1) gave essentially similar results. The thrombin was labelled with ¹²⁵I following the ICI method of McFarlane as developed for fibrinogen¹⁰. The labelled thrombin clotted fibrinogen and aggregated platelets in a way similar to unlabelled thrombin. The label was found to be unstable and the sample was dialysed against 0.1 M Tris-0.1 M NaCl, pH 7.2 before use. Because of this elution problem, the label simply serves the purpose of following the protein to which thrombin might bind and is not suitable for quantitative estimations at this stage. The activity of thrombin was measured from clotting time of human fibrinogen (Grade L, Kabi, Sweden) with respect to a standard preparation.

All experiments were done in duplicate and the data presented are average values. To 10–100 μl of labelled thrombin in each plastic conical centrifuge tube, 10 ml of washed platelets in modified Tyrode's solution was added. The tubes were capped, rapidly inverted once and then held at room temperature for 15 min. Depending on the experimental conditions and the amount of thrombin present, the platelets in some of the tubes aggregated. The samples were centrifuged in a swing out rotor (International Centrifuge Co Model CS, Massachusetts) at 2,500 r.p.m. for 10 min. The platelet sediments were washed once with physiological saline in the same conditions. Preliminary experiments showed that further washing did not make any significant difference in the results. The supernatant, the wash and the platelets were counted in a Packard Auto Gamma Spectrometer. A significant amount of radioactivity was found to be bound to the platelets (Fig. 1). It is interesting to note that a saturation point was not reached within the range studied even though aggregation occurred at a much lower concentration of thrombin. It seems that as the concentration of the added thrombin increases, the number of receptor sites also increases, suggesting transformation of the membrane with unmasking of new binding sites.

Previous studies with thrombin-sensitive proteins were generally done with platelets washed by the method of differential centrifugation. The platelets are fragile and it is now well recognised that this method of handling causes considerable and variable damage to the platelets. This

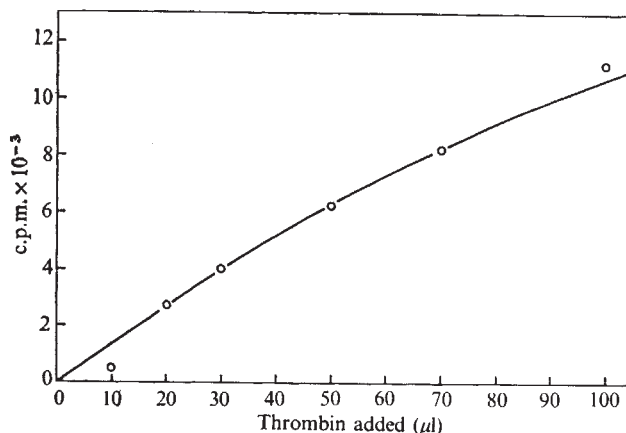


FIG. 1 Binding of thrombin by washed platelets. The platelets were isolated and washed by the metrizoate method and suspended in a modified Tyrode's solution at a concentration of approximately $30,000 \mu\text{l}^{-1}$. The labelled thrombin solution had 20 units ml^{-1} .

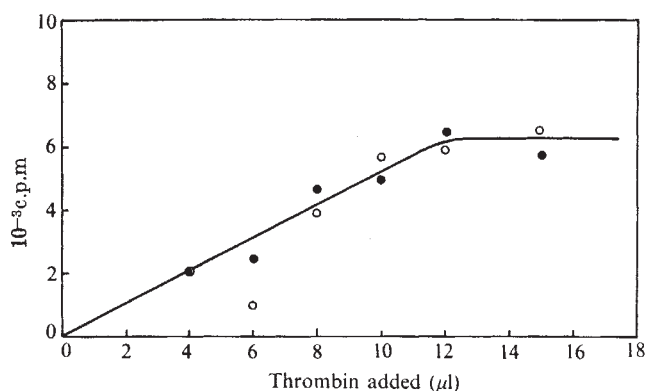


FIG. 2 Binding of thrombin by platelets in plasma. Platelet-rich plasma (10 ml) was incubated with different amounts of labelled thrombin. The platelets were collected by centrifugation, washed and counted. The thrombin solution has 30 units ml^{-1} . $\circ$, Control; $\bullet$, aspirin (1 mg ml^{-1} final concentration).

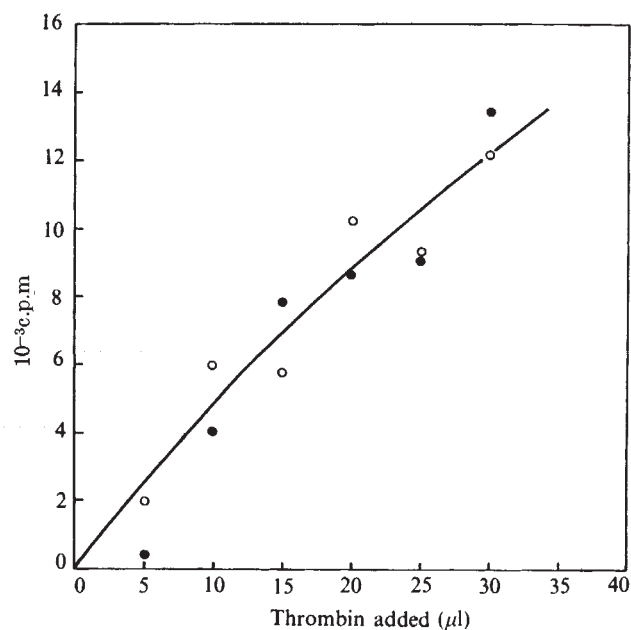


FIG. 3 Effect of PMSF on the binding of thrombin by platelets in plasma. The experimental procedure was similar to that for Fig. 2. The thrombin solution had 3 units ml^{-1} . $\circ$, Control; $\bullet$, PMSF (1 mg ml^{-1} final concentration).

might conceivably make normally inaccessible protein components available for nonspecific action of thrombin. Although the metrizoate method is more gentle than differential centrifugation, it was considered that it should be possible to extend these binding studies to platelets in plasma. There is a critical range, generally in the area of 0.2–0.8 units ml^{-1} final concentration, of thrombin which will aggregate the platelets without clotting fibrinogen. For each experiment, this critical value of thrombin was first determined by studying the aggregation behaviour of the platelets in plasma in an aggregometer. The PRP platelets were then treated with thrombin concentrations below this critical value.

The experimental procedure was similar to that described above for washed platelets, except that the time of incubation was 2 to 10 min. Again a substantial amount of thrombin was found to be bound to the platelets (Fig. 2). With platelets in plasma however, a saturation point was attained. Blood coagulation, haemostasis and thrombosis are complicated processes involving the interaction of several factors, a number of which are thrombin-sensitive. The possibility that additional receptor sites became available due to handling and washing of platelets should also be entertained. It was further argued that it should be possible to extend these binding studies to whole blood without any handling and separation. At this point, it is difficult to obtain precise quantitative data from these experiments because of technical problems in achieving clear separation between plasma, platelets and red cells. My results do, however, show that the platelets bind thrombin preferentially. On a per cell basis, the red cells bind little, if any, thrombin.

It is well known that aggregation of platelets with low concentrations of thrombin is inhibited by aspirin. It has also been reported that phenylmethylsulfonyl fluoride (PMSF) inhibits the clotting activity of thrombin although there is very little effect on the esterase activity⁹. Thrombin treated with PMSF fails to induce platelet aggregation. It was considered worthwhile to investigate the effect of these two inhibitors of platelet aggregation on the thrombin binding capacity of the platelets. From Figs 2 and 3, it can be seen that neither aspirin nor PMSF affect the binding of thrombin to platelets. It must therefore be concluded that binding of thrombin in itself is not sufficient to cause aggregation of the platelets and that one or more additional steps are involved. Similar results have been obtained using diisopropyl fluorophosphate (DFP) treated thrombin¹¹.

The platelet buttons from the above control experiments were suspended in 0.1 M Tris, 0.25 M NaCl, 1% Triton X-100, pH 7.5. The suspensions were vortexed repeatedly and then centrifuged at 12,000 g for 10 min. Most of the

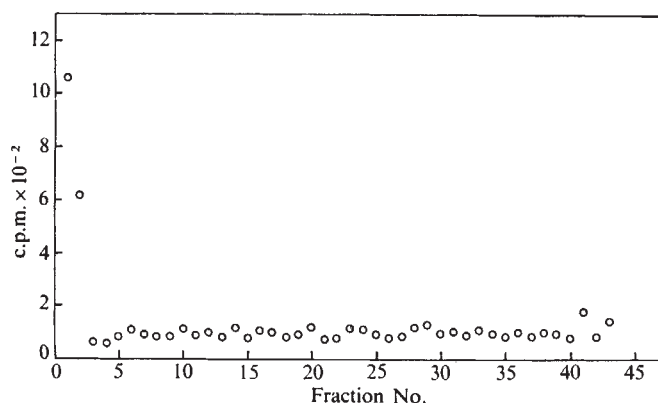


FIG. 4 Distribution of radioactivity after gel electrophoresis. Platelets were treated with labelled thrombin as in Fig. 1. The washed platelet button was dissolved in 0.05 M Tris, 0.25 M NaCl, 1% Triton X-100, pH 7.5 and electrophoresed in 5% acrylamide gel. The gel was cut into 2 mm sections and counted.

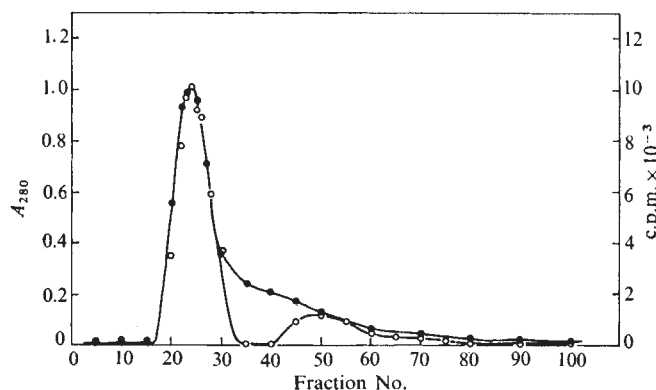


FIG. 5 Variation of absorbance (○) at 280 nm and of radioactivity (●) after gel filtration. Platelets were treated with labelled thrombin, washed and dissolved in Tris—NaCl—Triton X-100. After centrifugation and dialysis, the sample was applied to a column (2.5 cm × 40 cm) of Sephadex G-200 equilibrated with 0.05 M Tris, 0.25 M NaCl, 0.1% Triton X-100, pH, 7.5. Fraction size 2 ml.

platelet material went into solution, however, a small amount of sediment containing appreciable radioactivity was obtained. Here I deal with the soluble part only. The supernatant was analysed by disc electrophoresis at 5% or 7% gel concentrations. A single peak containing radioactivity was observed at the origin (Fig. 4). The stained gels also showed protein material in this area. Analyses of control samples showed that this peak is not due to thrombin itself. Several supernatant samples from the electrophoresis experiment were pooled and dialysed overnight against Tris—NaCl—Triton. The sample filtered through a column of Sephadex G-200 showed one main peak at void volume followed by a shoulder, containing radioactivity (Fig. 5).

The following conclusions may be drawn from these results. Human platelets bind thrombin tightly; this binding is specific and preferential compared with other blood components. Binding of thrombin *per se* is not sufficient for platelet aggregation and other steps are involved. With washed platelets, the number of receptor sites is dependent on the concentration of thrombin used indicating transformation of the membrane. In human platelets the receptor of thrombin seems to be a protein with a molecular weight of over 200,000.

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Vitamin B₁₂ antagonism by monocarboxylic acids and anilides of cyanocobalamin

VARIOUS test systems have been used to examine the anti-metabolite activity of vitamin B₁₂ analogues¹. In general, the tests have relied on the demonstration of growth inhibition or depression in either vitamin B₁₂-requiring microorganisms or vitamin B₁₂-deficient experimental animals. I have investigated the effect of the mixed isomeric monocarboxylic acids of cyanocobalamin and the anilides derived from these acids on the *in vivo* biochemical function of vitamin B₁₂ in vitamin B₁₂-depleted baboons.

Seven male baboons (*Papio cynocephalus cynocephalus*) weighing between 10 and 15 kg were used. They had been fed on a vitamin B₁₂-deficient diet consisting of starch, sucrose, vitamin-free casein and maize oil, with salt and vitamin supplements, and although they were haematologically normal, by comparison with baboons fed the same diet supplemented with 2 µg vitamin B₁₂ per baboon per day, they had low serum and liver vitamin B₁₂ levels and excreted increased quantities of methylmalonic acid (Table 1). The vitamin B₁₂-deficient diet was fed throughout the study, but the daily folic acid intake which had previously been 1 mg was reduced to 0.1 mg. The analogues were administered intramuscularly as sterile solutions in saline. The monocarboxylic acid was given to three baboons at a daily dose rate of 500 µg per kg body weight for 1 month and at a daily dose rate of 250 µg per kg body weight for a further 2 months. The anilide was given to one baboon at a daily dose rate of 250 µg per kg body weight for 3 months. The three remaining baboons served as controls and received daily injections of saline. Vitamin B₁₂ and folic acid levels were measured microbiologically with *Lactobacillus leichmannii*² and *casei*³ respectively. Methylmalonic acid (MMA) was measured by thin layer chromatography⁴ and formiminoglutamic acid (Figlu) enzymatically⁵. Haematology was carried out on a Coulter counter model ZBI.

Both analogues produced similar effects. The most marked effect was on the excretion of MMA. In vitamin B₁₂ deficiency there is an increase in the amount of MMA excreted in the urine due to a reduction in the tissue concentration of 5'-deoxyadenosyl-B₁₂ which is a cofactor in the conversion of methylmalonyl CoA to succinyl CoA⁶. Valine, a metabolic precursor of methylmalonyl CoA, causes an increase in the

amount of MMA excreted in vitamin B₁₂ deficiency⁷. Before treatment started, all seven baboons excreted increased amounts of MMA after a loading dose of 5 g L-valine given intraperitoneally. Treatment with either the monocarboxylic acid or the anilide caused an approximately ten-fold increase in the amount of MMA excreted (Fig. 1) and it remained higher than that in the control animals for some months after treatment stopped.

Apart from the isomerisation of methylmalonyl CoA, the only other reaction in mammalian tissues known to require vitamin B₁₂ is the transfer of a methyl group from N⁵-methyltetrahydrofolate to homocysteine to form methionine and tetrahydrofolate⁸. In vitamin B₁₂ deficiency serum folate levels tend to be high⁹ and there is an increase in the excretion of Figlu¹⁰. It has been proposed that the former is due to a build up of N⁵-methyltetrahydrofolate and the latter to the consequent reduction in the concentration of tetrahydrofolate which is required for the conversion of Figlu to glutamic acid. The amount of Figlu excreted after an oral dose of 5 g L-histidine increased in all three baboons treated with the monocarboxylic acid and also in the baboon treated with the anilide (Fig. 2). The increase, however, was only small and returned to normal soon after treatment stopped. The

TABLE 1 Comparative data for baboons fed vitamin B₁₂-deficient diet or vitamin B₁₂-supplemented diet

	Vitamin B ₁₂ -deficient (7)*	Vitamin B ₁₂ -supplemented † (3)*
Serum vitamin B ₁₂ (pg ml ⁻¹)	19 ± 3.7	219 ± 39.8
Serum folate (ng ml ⁻¹)	5.3 ± 0.59	5.9 ± 0.78
Liver vitamin B ₁₂ (µg g ⁻¹)	0.32 ± 0.03	1.07 ± 0.20
Methylmalonic acid excretion‡ (mg per 24 h)	24 ± 1.6	1 ± 0.4
Formiminoglutamic acid excretion§ (mg per 8 h)	20 ± 4.1	16 ± 1.5

The results are means ± standard errors.

* No. of animals

† 2 µg d⁻¹

‡ After 5 g L-valine given intraperitoneally

§ After 5 g L-histidine given orally

serum folate levels in the three baboons treated with the monocarboxylic acid were higher (3.2, 4.2 and 3.2 ng ml⁻¹) than in the three control animals (2.3, 2.4 and 2.2 ng ml⁻¹), but not in the baboon treated with the anilide (2.0 ng ml⁻¹).

The megaloblastic anaemia seen in vitamin B₁₂ deficiency in man has not so far been reproduced in vitamin B₁₂-deficient experimental animals. The haematological parameters of the baboons treated with either the monocarboxylic acid or the anilide remained unchanged during the first 2 months. At this time anaemia was induced by removing approximately 400 ml blood over a period of 7 d. Iron was administered intramuscularly to prevent iron deficiency. The rate of red blood cell regeneration was lower in the treated animals than in the controls (Fig. 3). There was, however, no increase in the mean cell volume suggesting that the mechanism by which the analogues depress the rate of red blood cell regeneration is not the same as that producing the anaemia of vitamin B₁₂ deficiency in man. Csanyi *et al.*¹¹ found that the administration of cyanocobalamin monocarboxylic acid produced leucopenia in rats. This was not so in my study; both the number of white blood cells and platelets remained normal.

The increase in the excretion of MMA suggests that both the monocarboxylic acid and the anilide exerted an antagonistic effect on the function of vitamin B₁₂. The results however shed no light on the mechanism of their action. One possibility is that they might compete with and displace vitamin B₁₂ from the tissues. This would result in an

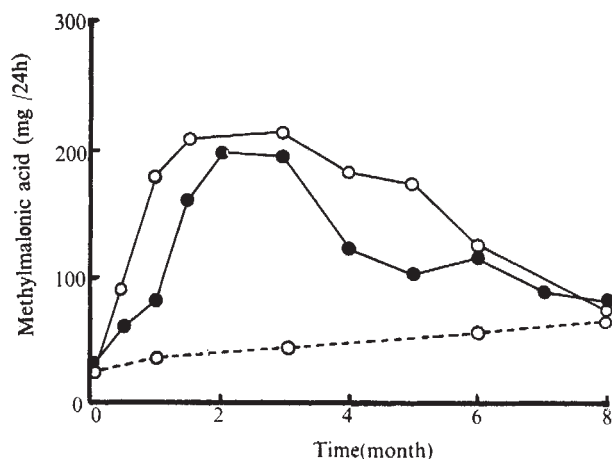


FIG. 1 Methylmalonic acid excretion after 5 g L-valine (intraperitoneal) by vitamin B₁₂-depleted baboons which were given intramuscularly for 3 months either the monocarboxylic acids (○—○, mean of three animals) or anilides (●—●) of cyanocobalamin; and in control baboons given saline (○—○, mean of three animals).

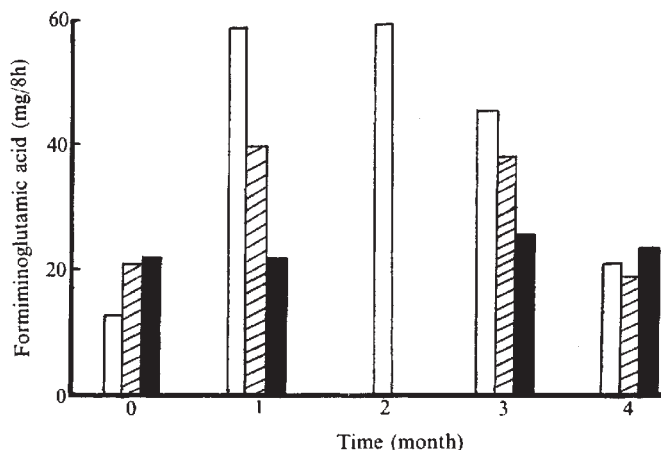


FIG. 2 Formiminoglutamic acid excretion after 5 g oral histidine by vitamin B₁₂-depleted baboons given intramuscularly for 3 months either the monocarboxylic acids (▨, mean of three animals) or anilides (□) of cyanocobalamin and in control baboons given saline (■, mean of three animals).

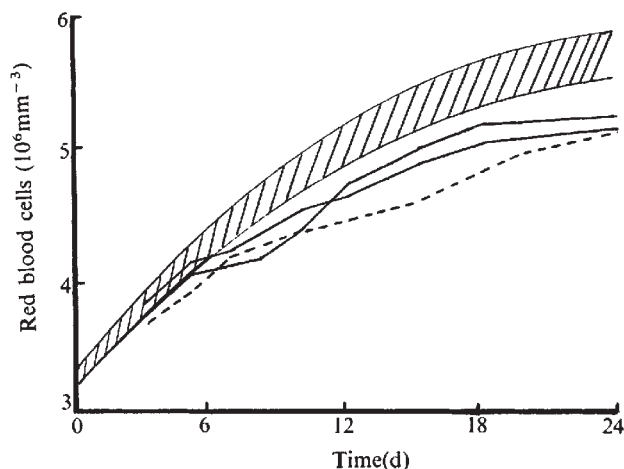


FIG. 3 Red blood cell regeneration in vitamin B₁₂-depleted baboons given intramuscularly either the monocarboxylic acids (—) or anilides (---) of cyanocobalamin. The shaded area represents the red blood cell regeneration in control baboons treated with saline.

increase in the serum vitamin B₁₂ level. Unfortunately, both analogues supported the growth of *L. leichmannii*, the micro-organism used for estimating vitamin B₁₂; 1 µg monocarboxylic acid caused growth equivalent to 0.16 µg vitamin B₁₂ and 1 µg anilide caused growth equivalent to 0.029 µg vitamin B₁₂. It was not surprising, therefore, to find a large increase in the apparent serum vitamin B₁₂ levels of the treated animals. The levels in the baboons treated with the monocarboxylic acid were greater than 2,000 pg ml⁻¹ and in the baboon treated with the anilide it was 323 pg ml⁻¹. Measurement of the concentration of vitamin B₁₂ in the liver, 3 months after treatment stopped, was also inconclusive since the levels in the three baboons treated with the monocarboxylic acid (0.18, 0.19 and 0.15 µg g⁻¹) and in the baboon treated with the anilide (0.21 µg g⁻¹) were similar to the levels in the control animals (0.15, 0.16 and 0.23 µg g⁻¹).

It has been suggested that the accumulation of methylmalonic acid may be responsible for the neuropathological changes seen in vitamin B₁₂ deficiency in man¹². Although I did not undertake detailed neurological studies, none of the

baboons developed any physical signs of neurological impairment.

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Group analysis of volatile and non-volatile N-nitroso compounds

THERE is much concern about the possible widespread occurrence of N-nitroso compounds in the environment¹. Many N-nitroso compounds are known to be carcinogenic^{2,3}, and some 'volatile' nitrosamines have been found in cured food products such as smoked fish⁴⁻⁶, cooked bacon (ref. 5 and United States Department of Agriculture News Release, 1972), and sausages⁵. N-nitroso compounds are generally unstable and have a wide range of physical properties, particularly with respect to volatility and solubility.

Analytical methods are available for the fourteen most volatile nitrosamines when they are present at the µg kg⁻¹ level in the original foodstuff⁷. The procedures involve concentration and clean up by distillation and extraction, followed by separation by gas-liquid chromatography (GLC) and detection by either the Coulson conductivity or the flame ionisation GLC detectors. Because they are non-specific to N-nitroso compounds, however, the GLC detectors are useful for screening purposes only; confirmation by GLC-mass spectrometry is still mandatory^{8,9}.

Judging from the wide variety of complex secondary amines occurring in nature, a large group of naturally occurring high molecular weight N-nitroso compounds must be expected. As a result of their high molecular weight, they would be 'non volatile', and not amenable to clean up by steam or vacuum distillation. For this reason methods are not yet available for the analysis of non-volatile N-nitroso compounds, even at mg kg⁻¹ levels.

Here we describe a group-specific N-nitroso compound analyser which is capable of detection at the µg kg⁻¹ level without preconcentration or purification. A detailed description of the experimental apparatus, including flow diagrams and associated electronic circuitry, is presented elsewhere¹⁰. The N-nitroso compound, dissolved in dichloromethane (DCM), is injected into a flash heater and swept by argon

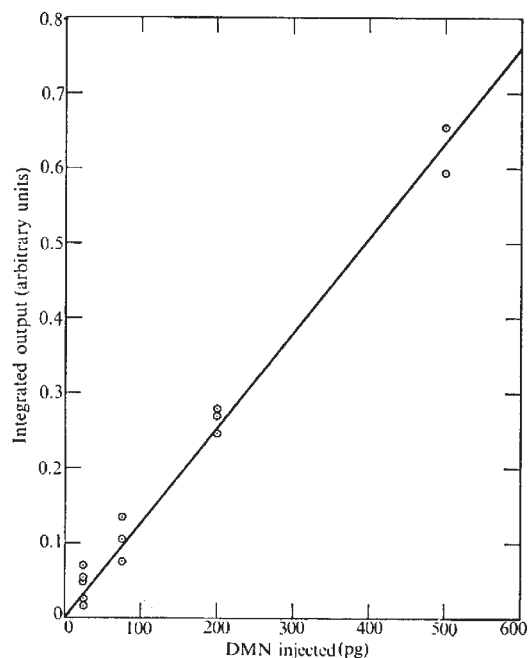


Fig. 1 Plot of integrated instrument response in arbitrary units versus picograms of DMN injected in a 5 μ l sample.

carrier gas into a catalytic pyrolysis chamber. The activated tungsten oxide catalyst selectively cleaves the N-nitroso compounds. Pyrolysis products and solvent then enter a reaction chamber, maintained at reduced pressure (3.5 torr) by a rotary vacuum pump. Ozone, also enters the reaction chamber, where it reacts with nitric oxide, giving excited nitrogen dioxide. The excited nitrogen dioxide rapidly decays back to its ground state with emission of light in the near infrared (0.6 to 2.8 μ m). Using an infrared-sensitive photomultiplier tube, we detect and quantitatively measure the intensity of the emission. A red cut-off filter is used to prevent the photomultiplier tube from responding to emissions at wavelengths shorter than 0.6 μ m. The output of the photomultiplier tube is amplified, the intensity of the light emission being directly proportional to the number of mol of N-nitroso compound introduced into the instrument.

A typical calibration curve for DMN is shown in Fig. 1 as a plot of instrument response against pg of DMN injected. At a signal to noise ratio of unity, 35 pg of DMN are required for detection, corresponding to a sensitivity limit of 0.4×10^{-12} mol of N-nitroso compound. The calibration remains linear over six orders of magnitude, from pg to μ g levels. A similar mol response has been found for a variety of volatile and non-volatile N-nitroso compounds, including ethyl-N-nitrososarcosinate, diethylnitrosamine, dibutylnitrosamine, diphenylnitrosamine, N-nitroso-N-ethyl-aniline, and phenylnitrosourea. For N-nitroso-methylurea, because of its extraordinary sensitivity to light, we found that most of it had already decomposed during the course of preparing the calibration solutions but the predicted mol response was obtained when the calibration solutions were prepared using blackened ampules.

A wide variety of compounds with structures similar to the N-nitroso compounds have been tested as 1% solutions in DCM for possible positive interference¹¹. No false positives, up to the limit of the instrument sensitivity, were detected.

To test the feasibility of using the analyser as a selective detector for N-nitroso compounds in complex mixtures, recovery experiments were carried out at the μ g kg⁻¹ level with DMN (a volatile N-nitrosamine) and diphenylnitrosamine (a non-volatile nitrosamine). Five grammes of fresh

TABLE 1 Recovery experiments of DMN, and diphenylnitrosamine from fresh beef and fresh herring at the μ g kg⁻¹ level.

Sample	Percentage recovery*
39 μ g kg ⁻¹ DMN in fresh beef	80%
110 μ g kg ⁻¹ DMN in fresh beef	77%
242 μ g kg ⁻¹ DMN in fresh beef	87%
20 μ g kg ⁻¹ DMN in fresh herring	85%
60 μ g kg ⁻¹ DMN in fresh herring	90%
120 μ g kg ⁻¹ DMN in fresh herring	97%
60 μ g kg ⁻¹ diphenylnitrosamine in fresh herring	100%
120 μ g kg ⁻¹ diphenylnitrosamine in fresh herring	75%

* Each experiment performed in duplicate.

ground beef or fresh herring were homogenised with 5 ml of DCM and transferred into a Teflon-capped centrifuge tube. The homogenised mixture was spiked with a known volume of a solution containing 1 μ g ml⁻¹ of the N-nitroso compound. The tube was shaken for 30 min and then centrifuged. Five microlitres of the bottom DCM layer was injected directly into the analyser, without further purification or concentration. The recovery for both the volatile and non-volatile N-nitroso compounds was in the range of 75 to 100% (Table 1). As the materials tested contain a vast number of different compounds, and considering that the materials were only extracted once, recoveries of this order are strong evidence that other compounds do not interfere with the detection of N-nitroso compounds by this method.

Because of the demonstrated specificity of the analyser to N-nitroso compounds, an instrument selective to individual N-nitroso compounds would be available if the N-nitroso compounds could be separated from each other before introduction into the analyser. Relatively crude separation would be required, for it is only necessary to separate N-nitroso compounds from each other, and not from the other substances present. Additional studies are currently under way to interface the analyser with GLC.

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Dissociability and tRNA content of monosomes produced by dimethylnitrosamine and starvation

DIMETHYLNITROSAMINE is an hepatotoxic agent which disaggregates liver polysomes into monosomes^{1,2}. Previous investigations have suggested that polysome disaggregation results from alkylation and subsequent fragmentation of mRNA in polysomes³, producing complexed ribosomes. But, fragmentation has been ruled out by the findings that monosomes produced by dimethylnitrosamine dissociate into subunits in solutions containing high concentrations of KCl^{4,5} and that pretreatment of animals with cycloheximide prevents polysome disaggregation by dimethylnitrosamine⁶. In bacteria the single ribosome population contains both complexed and runoff ribosomes which can be distinguished by their dissociability⁷ and tRNA content⁸. Therefore, we have compared these properties in monosomes produced by dimethylnitrosamine and by starvation, to determine whether they are runoff ribosomes. Our results show that in spite of their similar dissociability, monosomes produced by dimethylnitrosamine and starvation differ in tRNA content.

A single dose of dimethylnitrosamine (Fig. 1a) or 72 h of starvation (Fig. 1c) completely disaggregated liver polysomes into 80S monosomes which dissociated into 40S and 60S subunits in 0.3 M KCl (Fig. 1b and d). To characterise these ribosomes further, RNA extracted from monosomes was analysed by polyacrylamide gel electrophoresis and the ratios of 4S to 5S RNA were determined. Figure 2 shows a typical profile of RNA extracted from normal mouse liver polysomes (Fig. 2a and b), monosomes produced by dimethylnitrosamine (Fig. 2c and d), and monosomes produced by starvation (Fig. 2e and f). Under these conditions 18S and 28S RNA remained at the top of the gel whereas 4S and 5S RNA readily entered the gel. The 4S peak from normal polysomes was larger in samples in which RNA was extracted after incubation of polysomes with Pronase (Fig. 2a) than in samples in which RNA was extracted without previous incubation with Pronase (Fig. 2b). Kabat has demonstrated that a portion of the tRNA in polysomes carries nascent peptide chains and that this peptidyl-tRNA is extractable only after detachment of nascent peptide⁹.

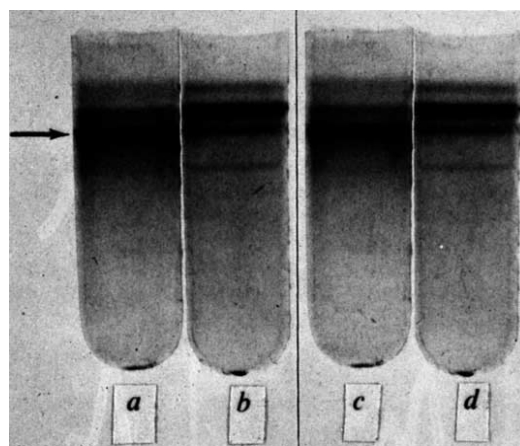


Fig. 1 Ribosome-polysome profiles of mouse livers in photopolymerised linear sucrose gradients. The arrow indicates the position of the 80S band. *a*, One hour after injection of dimethylnitrosamine, 25 mM KCl treatment; *b*, 1 h after injection of dimethylnitrosamine, 0.3 M KCl treatment; *c*, 72 h after starvation, 25 mM KCl treatment; *d*, 72 h after starvation, 0.3 M KCl treatment. Swiss albino male mice were either given a single intraperitoneal injection of 50 mg kg⁻¹ body weight of dimethylnitrosamine or were starved. One hour after injection or 72 h after starvation livers were removed and ribosome pellets were prepared as previously described⁴ except that the post-mitochondrial supernatant was centrifuged at 35,000 r.p.m. in a type 50 rotor through a cushion containing 1 M sucrose in addition to buffer. To determine the dissociability of monosomes the pellets were rinsed twice and resuspended in buffer containing 10 mM Tris-HCl (pH 7.4), 5 mM MgSO₄, and either 25 mM KCl or 0.3 M KCl to a final concentration of 50 A₂₆₀ U ml⁻¹. Samples of 0.2 ml were layered onto 0.5–1.2 M linear sucrose gradients containing acrylamide gel components⁴. Gradients were centrifuged at 35,000 r.p.m. in a Spinco Model L centrifuge with a SW 50.1 rotor for 90 min at 5° C and then photopolymerised, sliced, and stained^{4,14}.

Therefore, all species of tRNA bound to ribosomes (peptidyl-tRNA, aminoacyl-tRNA and tRNA) are detected in samples previously incubated with Pronase, whereas only tRNA lacking peptide is detected in samples extracted with-

TABLE 1 No. of tRNA molecules on polysomal and single ribosomes

	4S:5S RNA ratio extracted with Pronase	4S:5S RNA ratio extracted without Pronase
Normal polysomal ribosomes	1.7	0.9
Dimethylnitrosamine monosomes	1.7	0.9
Starved monosomes	0.7	0.7
Dimethylnitrosamine monosomes (1.5 mM MgSO ₄)	1.6	0.7
40S subunit from dimethylnitrosamine monosomes	0	—
60S subunit from dimethylnitrosamine monosomes	1.4	—
Starved monosomes with normal supernatant	0.7	0.7
Dimethylnitrosamine monosomes in 1 M Tris (pH 9) for 4 h	1.7	1.4
Dimethylnitrosamine monosomes in 1 M Tris (pH 9) for 8 h	1.7	1.6
Dimethylnitrosamine monosomes in 1 M NH ₄ OH for 1 h	1.7	1.4
Dimethylnitrosamine monosomes in 1 M NH ₄ OH for 2 h	1.7	1.5
Dimethylnitrosamine monosomes in 1 M NH ₄ OH for 4 h	1.7	1.7
Dimethylnitrosamine monosomes with puromycin	1.5	1.4

Since each ribosome contains one molecule of 5S RNA the number of tRNA molecules per ribosome was determined from the molar ratio of 4S to 5S RNA⁹. Monosome and polysome pellets were prepared in buffer containing 3 mM MgSO₄ unless otherwise indicated. RNA was extracted from these pellets and analysed by electrophoresis as described in Fig. 2. Each peak was weighed and the 4S to 5S molar ratio was calculated by multiplying the value of the 4S peak by 1.5 and dividing by the value of the 5S peak⁹. RNA was extracted from isolated subunits obtained by dissociation of monosomes in 0.3 M KCl and centrifugation in sucrose gradients containing 0.3 M KCl for 4 h at 35,000 r.p.m. in a SW 50.1 rotor. Gradients were scanned at 260 nm and the 40S and 60S peaks were collected. Ribosome subunits were precipitated by the method of Dessev and Grancharov¹⁸. In some cases monosome pellets from livers treated with dimethylnitrosamine were resuspended in 1 M Tris-HCl (pH 9) or in 1 M hydroxylamine (pH 7)¹¹. Sodium dodecyl sulphate (6 mg ml⁻¹) was added and the samples were incubated at 30° C for the time indicated. Other ribosome pellets were resuspended in 50 mM Tris-HCl (pH 7.4), 0.5 M KCl, and 3 mM MgSO₄ and puromycin-HCl was added to a concentration of 1 mM. The samples were incubated for 30 min in ice and for 20 min at 35° C (ref. 12). RNA was extracted directly from the incubation mixtures. Postribosomal supernatant was prepared by centrifuging postmitochondrial supernatant from normal liver prepared in 50 mM Tris-HCl (pH 7.4), 100 mM KCl, and 3 mM MgSO₄ at 35,000 r.p.m. for 90 min in a type 50 rotor.

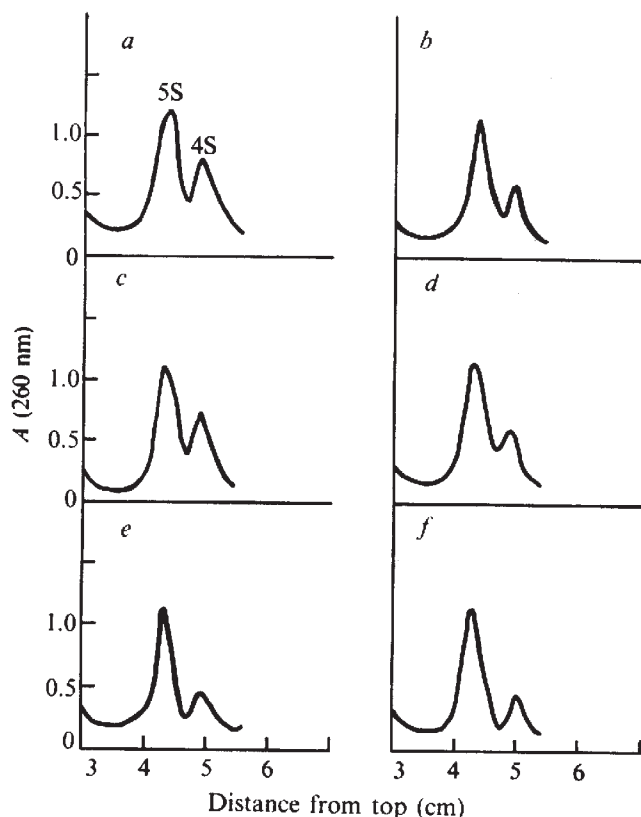


FIG. 2 Electrophoresis of RNA extracted from polysomes and monosomes. The peaks indicate the position of 5S and 4S RNA in the acrylamide gel as measured from the top of the gel. *a*, Normal polysomes with Pronase treatment; *b*, normal polysomes without Pronase treatment; *c*, monosomes 1 h after injection of dimethylnitrosamine with Pronase treatment; *d*, monosomes 1 h after injection of dimethylnitrosamine without Pronase treatment; *e*, monosomes 72 h after starvation with Pronase treatment; *f*, monosomes 72 h after starvation without Pronase treatment. Livers were homogenised in 50 mM Tris-HCl (pH 7.4), 100 mM KCl, and 3 mM MgSO₄ and 0.2 ml of postmitochondrial supernatant was centrifuged for 90 min in 0.5–1.2 M linear sucrose gradients in the same buffer. Gradients were scanned at 260 nm¹⁶. The remainder of the postmitochondrial supernatant was centrifuged through a 1 M sucrose cushion in the same buffer. RNA was extracted from ribosome pellets by the chloroform-isoamyl alcohol method of Oda and Joklik¹⁶. In samples from dimethylnitrosamine-treated and starved animals RNA was extracted only from monosome pellets in which gradient centrifugation revealed complete disaggregation of polysomes into monosomes. Microscopy of nucleolar segregation was also used to ascertain the toxic effect of dimethylnitrosamine¹⁷. Monosome pellets were resuspended in 5 mM Tris-HCl (pH 7.4) and the suspension was extracted immediately or was incubated for 1 h at 30° C with 3 mg ml⁻¹ Pronase and 4 mg ml⁻¹ sodium dodecyl sulphate⁹. RNA was precipitated overnight at -18° C with two volumes of ethanol and 0.2 M NaCl. RNA was analysed by electrophoresis in 4% acrylamide gels at 5 mA per gel for 65 min in 36 mM Tris-phosphate (pH 7.8), 30 mM Na₂HPO₄, and 1 mM EDTA as described by Kabat⁹. Gels were scanned at 260 nm by a Gilford spectrophotometer with a linear transporter. RNA (350 ug) was applied to each gel. *E. coli* tRNA was used to estimate the position of the 4S and 5S RNA peaks.

out previous proteolysis. Figure 2 further demonstrates that the RNA profile from monosomes produced by dimethylnitrosamine resembles the profile from normal polysomes. The 4S to 5S RNA ratio is substantially increased on Pronase preincubation (Fig. 2*c* and *d*). Monosomes from starved liver contained a smaller quantity of tRNA (Fig. 2*e* and *f*). Measurement of the 4S and 5S peaks from samples which had been treated with Pronase before extraction yielded

about 1.7 tRNA molecules per ribosome for normal polysomes and monosomes produced by dimethylnitrosamine but only 0.7 tRNA molecule per ribosome for monosomes produced by starvation (Table 1). However, polysomes and monosomes produced by dimethylnitrosamine contained only 0.9 molecule of tRNA in samples extracted without Pronase treatment. The ratio of 4S to 5S RNA for monosomes produced by starvation did not change. Thus, polysomes and monosomes produced by dimethylnitrosamine contain approximately 0.8 molecule of peptidyl-tRNA and 0.9 molecule of tRNA or aminoacyl-tRNA. Cornwell¹⁰ has reported that normal liver polysomes contain 1.5 tRNA molecules per ribosome.

To discount the possibility that the tRNA on monosomes produced by dimethylnitrosamine was the result of non-specific adsorption of tRNA to ribosomes, monosomes were prepared in buffer containing 1.5 mM MgSO₄. (Davis demonstrated that buffer containing a low concentration of magnesium reduces the amount of non-specifically bound tRNA⁸.) Under these conditions monosomes dissociated into 40S and 60S subunits but still retained 1.7 and 0.7 tRNA molecules per ribosome in samples treated with and without Pronase, respectively. Extraction of RNA from isolated subunits revealed that tRNA remained bound to the 60S subunit after dissociation (Table 1). Furthermore, when monosomes produced by starvation were mixed with post-ribosomal supernatant from normal liver and centrifuged through 1 M sucrose in buffer containing 3 mM MgSO₄, the ribosomes still contained only 0.7 tRNA molecule per ribosome both with and without Pronase treatment (Table 1). Therefore, the tRNA present on monosomes is probably not due to non-specific binding of tRNA.

If the difference in the ratios of 4S to 5S RNA with and without proteolysis was due to the presence of peptidyl-tRNA on ribosomes, then exposure of ribosomes to conditions which promote cleavage of nascent peptide from peptidyl-tRNA should have given the same ratio of 4S to 5S RNA whether or not the sample was incubated with Pronase before extraction. Table 1 shows that the difference between ratios in samples extracted with and without Pronase decreased as monosomes produced by dimethylnitrosamine were incubated at 30° C for increasing periods in the presence of 1 M Tris-HCl (pH 9) or 1 M hydroxylamine (pH 7). The ratios were nearly identical after incubation in Tris-HCl for 8 h or in hydroxylamine for 4 h (Table 1). These conditions enhance cleavage of nascent peptide from peptidyl-tRNA¹¹. Similarly, incubation of monosomes produced by dimethylnitrosamine with 1 mM puromycin at high ionic strength according to Blobel and Sabatini¹² eliminated the difference between ratios of 4S to 5S RNA extracted with and without Pronase treatment (Table 1).

Therefore, the monosomes which accumulate during starvation are likely to be runoff ribosomes since they dissociate into subunits and are free of peptidyl-tRNA⁷⁻⁹. The monosomes which accumulate after a single dose of dimethylnitrosamine, however, are unlikely to be runoff ribosomes or complexed ribosomes since they dissociate into subunits like runoff ribosomes, but contain peptidyl-tRNA and tRNA (aminoacylated and free tRNA) like complexed ribosomes. Complexed ribosomes do not dissociate into subunits under these ionic conditions⁴. Other investigators have demonstrated that only ribosomes free of mRNA dissociate into subunits, whereas ribosomes still attached to mRNA do not dissociate even if stripped of nascent peptide¹³. Therefore, the difference in dissociability between complexed ribosomes and monosomes produced by dimethylnitrosamine suggests that the latter monosomes are free of mRNA. These results suggest that dimethylnitrosamine disaggregates liver polysomes by promoting the premature fall off of ribosomes from mRNA during elongation.

Although the mechanism of polysome disaggregation by dimethylnitrosamine has not been ascertained, this study suggests that *in vivo* it is not fragmentation of mRNA or inhibition of initiation.

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Multiple forms of SS → DS RNA polymerase activity in reovirus-infected cells

THE genome of reovirus consists of a unique set of ten segments of double-stranded (DS) RNA¹⁻³. This DS RNA is replicated in infected cells on a preformed single-stranded (SS) RNA template which has the same polarity as messenger RNA⁴⁻⁷.

To gain insight into the mechanism by which the 10 DS RNA segments are assembled and encapsidated during viral morphogenesis we are studying the properties of the enzymes responsible for the synthesis of DS RNA in infected cells. Recently we described the isolation of a SS RNA-dependent DS RNA polymerase (SS → DS RNA polymerase) from infected L cells which is associated with virion-like particles⁸. Since the DS RNA synthesised by these particles remains associated with them and since DS RNA is synthesised on a preformed SS RNA template, we suggested that the ten segments of SS RNA associate with viral proteins in a virion-like particle and that DS RNA is synthesised within these particles⁸.

We have extended these studies and have identified several classes of subviral particles which possess SS → DS RNA polymerase activity. Each catalyses the synthesis of different

size classes of DS RNA segments.

A cytoplasmic extract was prepared from infected cells

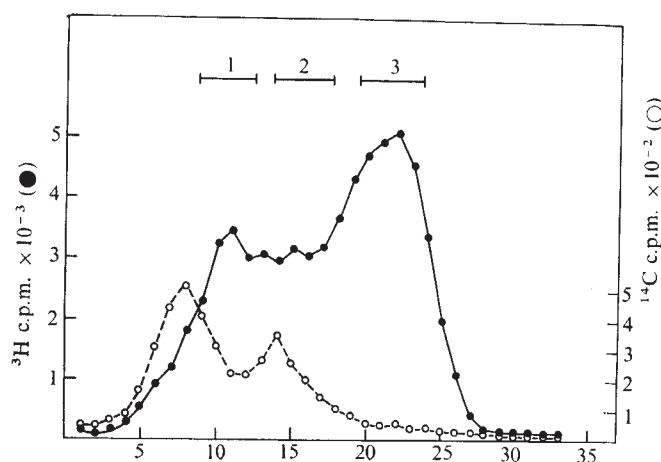


FIG. 1 Distribution of SS → DS RNA polymerases in glycerol-density gradients. 2×10^8 infected spinner cells maintained at 31° C and collected 18 h post infection⁷, were resuspended in 5 ml 0.01 M NaCl-0.1 M Tris, pH 8.0 (S-T buffer) and dounced in the presence of 1% Triton X-100 and 1% DOC. Nuclei were removed by centrifugation at 1,500 r.p.m. for 5 min. All procedures were performed at 4° C. The supernatant was layered over a 4 ml glycerol cushion (40% in 10^{-3} M Tris, pH 8.0) and centrifuged in a SW 41 rotor for 3 h at 38,000 r.p.m. The pellet was resuspended by sonication in 0.7 ml S-T buffer mixed with a small sample of 14 C-labelled purified reovirions and top component, and layered onto a 20–40% glycerol (in 10^{-3} M Tris, pH 8.0) gradient. The gradient was centrifuged at 30,000 r.p.m. for 1 h in a SW 41 rotor. Fractions were collected, diluted with an equal volume of 2 × S-T buffer and 0.25 ml of each fraction was assayed for SS → DS RNA polymerase activity by the addition of 0.1 μ mol each of ATP, GTP, and CTP, 6×10^{-3} μ mol UTP, 5 μ Ci 3H-UTP (15 Ci-mmol⁻¹), 0.9 μ mol MgCl₂, 0.9 μ mol β -mercaptoethanol and 0.1 μ g actinomycin D. After incubation for 1 h at 39° C, 0.2 μ mol cold UTP was added. After addition of NaCl to a final concentration of 0.15 M and 20 μ g-ml⁻¹ pancreatic ribonuclease, samples were incubated at 37° C for 30 min. The samples were precipitated with an equal volume of 10% trichloroacetic acid and the precipitate collected on Millipore filters. Radioactivity was determined by liquid scintillation counting⁸. Sedimentation is from the right to the left in the Figure. 22% of the total polymerase activity was recovered in the pellet of the gradient. The sedimentation coefficients of purified virions and top component are 630 S and 470 S respectively. ●—●, SS → DS RNA polymerase products; ○—○, 14 C virion and top component.

maintained at 31° C and collected 18–20 h after infection, when RNA synthesis is at a maximum. All SS → DS RNA polymerase activity in this extract was pelleted by centrifugation for 3 h at 35,000 r.p.m. When the material in this pellet was centrifuged in a glycerol gradient and the fractions assayed for SS → DS RNA polymerase activity, a broad spectrum of polymerase activity was observed (Fig. 1). Reproducibly, two peaks of polymerase activity were found which correspond to structures with approximate sedimentation coefficients of 550 and 250S in comparison with virions (630S) and top component (470S). The major polymerase peak always sedimented at 250S. Approximately 20% of the polymerase activity was recovered in the pellet of the gradient. No loss in polymerase activity was observed after gradient centrifugation, when compared with the enzyme activity in the cytoplasmic pellet before centrifugation.

To investigate the properties of the SS $\rightarrow$ DS RNA polymerase activities, fractions were combined as shown in Fig. 1 and designated DS RNA polymerase 1, 2 and 3 respectively. The band of enzyme activity sedimenting at approximately 550S (DS RNA polymerase 1) is the enzyme described previously⁸.

The DS RNA products of each polymerase were analysed on sucrose density gradients and polyacrylamide gels to determine whether the three polymerases synthesise all ten species of DS RNA. Viral DS RNA is separated into three size classes, L, M and S, in sucrose-density gradients^{1,2}. As shown in Fig. 2, the three SS $\rightarrow$ DS RNA polymerases synthesise the three size classes to different extents. DS RNA polymerase 1 synthesises predominantly DS RNA of the L class (Fig. 2a), DS RNA polymerase 2 synthesises the M class in excess over L and S DS RNA (Fig. 2b), and DS RNA polymerase 3 predominantly the S class (Fig. 2c).

Further separation of the viral DS RNA into ten species is obtained by polyacrylamide gel electrophoresis³ (Fig. 3d). DS RNA polymerase 1 synthesises all ten species of DS RNA, but the predominant species are the three L species (Fig. 3a). Again DS RNA polymerase 2 synthesises the three M species in relative excess (Fig. 3b) and DS RNA polymerase 3 synthesises a relative excess of the four S species (Fig. 3c).

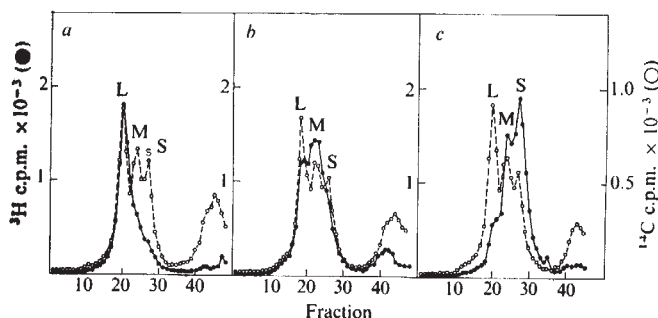


FIG. 2 Sedimentation of the DS RNA products of the three SS $\rightarrow$ DS RNA polymerases in sucrose-LiDS gradients. DS RNA polymerases 1, 2, and 3 were isolated from a glycerol gradient by combining fractions as described in Fig. 1 and incubated with the components of the polymerase reaction mixture. After the reaction, RNA was extracted with phenol, precipitated with ethanol and resuspended in $2 \times$ SSC. After the addition of ^{14}C -DS RNA extracted from purified virions⁹, the mixture was incubated for 15 min at 37°C with $5 \mu\text{g ml}^{-1}$ pancreatic RNase⁸. After the addition of LiDS to 1%, the sample was layered on a 15–30% sucrose-LiDS gradient and centrifuged in a SW 27 rotor for 24 h at 25,000 r.p.m. Fractions were collected and precipitated with an equal volume of 30% trichloroacetic acid in the presence of bovine serum albumin ($10 \mu\text{g ml}^{-1}$). Sedimentation is from right to left, a, b, and c represent the products of SS $\rightarrow$ DS RNA polymerases 1, 2, and 3 respectively. $\bullet$ — $\bullet$, ^3H -labelled SS $\rightarrow$ DS RNA polymerase products; $\circ$ — $\circ$, ^{14}C -DS RNA extracted from purified virions.

In virions, the ten DS RNA segments are surrounded by two protein layers. The outer protein layer can be removed by chymotryptic digestion to produce a core which contains the genome and the inner capsid proteins. This structure has a buoyant density in CsCl of 1.43 g cm^{-3} as compared with 1.36 g cm^{-3} for complete virions⁹.

We have suggested previously that DS RNA polymerase 1 consists of a core-like structure to the outside of which additional protein molecules are attached and that DS RNA is synthesised within this particle⁸. The evidence for this is as follows: the enzyme sediments slightly slower than

virions in sucrose-density gradients, its buoyant density is very close to that of virions (1.34 g cm^{-3} compared with 1.36 g cm^{-3} for virions) and after chymotrypsin treatment its density increases to that of viral cores (1.43 g cm^{-3}). Furthermore, DS RNA synthesised by these particles is not released but remains associated with structures which have the same sedimentation properties in sucrose and caesium gradients as the enzyme itself.

Similar experiments were undertaken to determine whether DS RNA polymerases 2 and 3 are also associated with sub-viral particles. DS RNA polymerases 2 and 3 resemble DS RNA polymerase 1 in that their buoyant densities are also 1.34 g cm^{-3} . In the case of DS RNA polymerase 3, a second but minor enzymatic activity was sometimes detected which had an approximate buoyant density of 1.43 g cm^{-3} . It was not possible to determine the effect of chymotrypsin treatment on the densities of DS RNA polymerases 2 and 3, since both lost their enzymatic activity after chymotrypsin treatment.

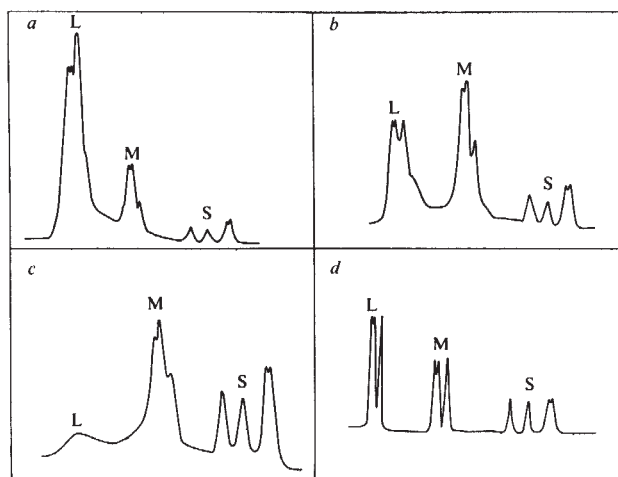


FIG. 3 Polyacrylamide-gel electrophoresis of the DS RNA products of DS RNA polymerases 1, 2, and 3. DS RNA polymerases 1, 2, and 3 were incubated under standard reaction conditions except that ^3H -UTP was replaced by ^{32}P -UTP ($10 \mu\text{Ci}$ per 0.25 ml). RNA was extracted with phenol, treated with $5 \mu\text{g}$ RNase in $2 \times$ SSC (see legend to Fig. 2) and electrophoresed on 7.5% polyacrylamide slab gels, as described previously¹⁰. ^{14}C -labelled DS RNA extracted from purified virions was also resuspended in $2 \times$ SSC and treated with RNase before electrophoresis. Autoradiograms were prepared from these gels and scanned in a Gilford recording spectrophotometer¹⁰. a, b, and c, products of DS RNA polymerase 1, 2, and 3 respectively; d, DS RNA extracted from purified virions.

Similarly, DS RNA polymerases 2 and 3 resemble DS RNA polymerase 1 in that DS RNA synthesised *in vitro* remains associated with the enzyme complexes. In the case of DS RNA polymerase 2, newly made DS RNA is associated with structures with a buoyant density of 1.34 g cm^{-3} which increases to 1.43 g cm^{-3} after chymotrypsin treatment. The DS RNA polymerase 3 product also has a buoyant density of 1.34 g cm^{-3} , but 50 to 75% of these structures are completely degraded by chymotrypsin treatment. Infrequently, the DS RNA polymerase 3 product contained a second particle with a buoyant density of 1.43 g cm^{-3} . This may correspond to particles described by Sakuma and Watanabe¹¹. Possibly this is a product of the second particle sometimes found in DS RNA polymerase 3 preparations which also has a density of 1.43 g cm^{-3} .

In view of these findings we propose that ten segments of SS RNA associate with viral proteins to form a particle which can only replicate a limited number of SS RNA species. Before replication of other SS RNA species can occur, certain sequential changes in the structure of such particles are necessary. These changes would result in particles more closely resembling virions as judged by increased resistance of the inner proteins to chymotryptic digestion and altered sedimentation rates. One of the first particles formed is probably DS RNA polymerase 3, since this particle resembles virions the least.

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pH, Salinity and Temperature Tolerance of Lake Magadi *Tilapia*

THERE are several species of *Tilapia* isolated in the alkaline lakes of the Great Rift Valley of Africa, living in extreme conditions of temperature, salinity and pH. One of these fish, the Lake Magadi *Tilapia* (*Tilapia grahami*), lives in the lagoons and alkaline volcanic springs around the margin of Lake Magadi in Kenya¹. The main salts of the springs, and the lake, are carbonate and bicarbonate of sodium.

To continue with studies on osmoregulation and acid-base balance in *Tilapia grahami*, we made some observations on its tolerance to changes in pH, salinity and temperature. Each of the three series of experiments was carried out on different fish as were the individual experiments within each series. Each experiment involved four to six medium-sized fish 3 to 5 cm long, which were kept in a glass jar. The fish were caught in nets in a lagoon (Fish Springs¹) and were transported to Nairobi in water taken from the same lagoon, which had pH values of 9.8 to 10.1, osmolarity of 518 to 600 mOsm per kg H₂O and a temperature, measured on different days, of 35.2 to 38.0° C.

In Nairobi, some fish were transferred to tap water, which

was well tolerated. Others were transferred to dilute solutions of hydrochloric acid of pH 3 to 6 or sodium hydroxide (of pH 8 to 12) in tap water. Those exposed to a pH of 3 to 4 or a pH of 12 died within 2 to 6 h. Fish kept at pH values between 5 and 11 showed no ill effects after 24 h. Throughout the experimental period the pH was regularly measured and adjusted by adding acid or base. Most experiments of this type were performed at 22° to 23° C, but similar pH tolerance limits were found in a few fish studied at 35° to 36° C during the first day after they had been brought from the lagoon.

Tolerance to salinity changes was tested at 22° to 23° C in 2 to 6% solutions of sodium chloride in tap water. Fish kept in 2 to 3% solutions were apparently in good condition after 24 h, those in 4% solution were alive after 8 to 10 h but died soon afterwards. Those maintained in greater concentrations died within a few hours.

Temperature tolerance was tested in fish kept in water from the lagoon. At 10° to 12° C the fish died within 1 to 2 h, but they survived after 24 h at 16° C. Temperatures of 22° to 23° C were well tolerated for several days. The upper limit for temperatures tolerated for at least 24 h seemed to be about 40° C. If the fish had been kept at 22° to 23° C for some days, however, immediate transfer to water of moderately high temperatures (35° to 36° C) was lethal.

Our study indicates that the ability of *T. grahami* to survive in alkaline waters is superior to that known for any other fish, while acid waters (pH 5) and salinity changes have been shown to be equally well tolerated by adult individuals of several freshwater and euryhaline species². The range of thermal tolerance of *T. grahami* is less than that previously demonstrated in the desert pupfish, *Cyprinodon*³, but the upper lethal temperatures are about the same. Previous observations have indicated that individuals of *T. grahami*, particularly brooding females, during their movements between the different pools may survive transient exposure to water temperatures of 40° to 44° C (compare refs 1 and 4).

In addition to high pH, salinity and temperature, the Lake Magadi *Tilapia* seems to be faced with low levels of dissolved oxygen. Preliminary measurements, obtained with the azide modification of the iodometric method⁵, showed oxygen levels of 1.1, 2.2 and 3.3 mg l⁻¹ in water from outlets of hot spring, main lagoon and shallow pool, respectively. Fish were observed in all these locations. Those used in our experiments were caught in the main lagoon; large individuals seemed to prefer the more shallow pools. Large amounts of free carbon dioxide also seem to be well tolerated as the fish showed no ill effects when kept in water from the lagoon while the water was acidified to pH 5 by dropwise addition of hydrochloric acid. Addition of strong acids to waters with a high bicarbonate content can produce concentrations of carbon dioxide which are fatal to other species of fish².

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IQ and Race

JENSEN has argued that the undoubted differences in mean IQ scores between racial groups in the United States reflect differences in genetic endowment. But a major problem in the interpretation of these differences is the difficulty of studying environmental and genetic effects separately. Each race tends to have, for historical reasons, characteristic child-rearing practices which are to some extent independent of social class. Hence the argument that because black children tend to have lower IQs than white children of the same social class they are genetically inferior is unconvincing; the inferior test scores could result from differences in the microenvironment of the family, such as linguistic practices and attitudes to achievement.

My colleagues and I have recently had an opportunity to study children of different racial origins reared in an identical environment. These were illegitimate children admitted to residential nurseries as infants until such time as their mothers could look after them or they could be found adoptive homes. All those tested had been healthy, full-term babies: the residential nurseries, run by three voluntary societies, were in

sixty-four such children: twenty-five were still in institutions, twenty-four had been adopted into white families at a mean age of 3.1 yr, and fifteen had been restored to their mothers (all but one of whom were white) at a mean age of 3.5 yr². Table 2 shows that the mean IQs of the different racial groups in each environment were very similar.

These studies were not undertaken with the aim of comparing the scores of different racial groups, but rather to examine the effects of different environments on development. In the first study children in nurseries where the quality of the staff talk to children (as assessed in observations) was highest had mean language comprehension scores one and a half standard deviations above those of children in the nurseries where the quality of staff talk was lowest. In the second study the adopted children had significantly higher mean IQs than either the children still in nurseries or those restored to their mothers, and these differences were related to measured aspects of the children's environment.

In both studies genetic aspects were neither controlled nor adequately known, that is, the children had not been randomly assigned to different environments and no parental IQs were available. It could therefore be argued that the

Table 1 Test Scores of Eighty-five Residential Nursery Children

	Reynell comprehension *			Reynell expression *			Minnesota non-verbal†		
	N	Mean	s.d.	N	Mean	s.d.	N	Mean	s.d.
White children	39	102.6	10.0	39	98.5	11.2	24	101.3	11.1
Black children‡	22	106.9	8.7	22	97.8	8.5	15	105.7	7.4
Mixed race children§	24	105.7	10.2	24	99.3	8.7	15	109.8	7.2
White against non-white		t=1.70, n.s.			t=0.02, n.s.			t=2.51, P<0.02	

* Converted to a mean of 100, s.d. 10.

† Only given to children aged 3 yr and older.

‡ Children with two West Indian or African parents.

§ Children with one West Indian or African parent and one white parent.

Table 2 Wechsler Pre-school and Primary Scale of Intelligence Full Scale IQs of Sixty-four Children Aged 4.5 yr

	N	Institutional		N	Adopted		N	Restored to mother	
		Mean	s.d.		Mean	s.d.		Mean	s.d.
White children	10	101.2	10.7	17	113.0	7.8	9	98.2	10.8
Mixed race children	7	109.3	8.2	7	119.9	16.6	5	102.2	12.3
Black children	8	105.6	10.3	0			1	106.0	
White against non-white		t=1.55, n.s.			t=1.45, n.s.			t=0.80, n.s.	

many respects of high quality, with a generous staff-child ratio and a plentiful supply of toys and books. Because the nurseries tended to be isolated the children did not meet community colour prejudice, and within the nurseries we saw no sign of such prejudice.

In the first study we tested all the healthy children in thirteen residential nursery groups who had been there at least six months and who were aged 2 yr 0 month-4 yr 11 months. Thirty-nine children had two white parents, twenty-two had two West Indian or African parents, and twenty-four were racially mixed. Seventy per cent had been admitted before their first birthday, and 86% before their second¹. The children were tested with a non-verbal intelligence test and a test of language comprehension and expression. Table 1 shows that the mean test scores of the different racial groups were very similar, and the only significant difference was in favour of the non-white children.

In the second study we tested all the available children aged 4.5 yr who had been admitted to any of the residential nurseries of these voluntary societies by the age of 4 months and remained there until at least the age of 2 yr. We found

non-white parents may have been of higher IQ than the white parents, or that children of higher IQ parents had been adopted or placed in nurseries where the staff talk was of high quality. We found no evidence to support such hypotheses. Children were placed in whichever nursery had a vacancy, and no attempt was made to assess the IQ of the children or their natural parents before placing them for adoption. The occupation of a third of the natural fathers was unknown, but for the rest there was no significant difference between the proportions of manual and non-manual workers in the different racial groups or different home or nursery environments. On the other hand, in both studies the relationship between the children's test scores and measured aspects of the environment was shown to be large and significant.

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book reviews

Intuitive science

Thematic Origins of Scientific Thought: Kepler to Einstein. By Gerald Holton. Pp. 495. (Harvard University: Cambridge, September 1973.) \$10 cloth; \$3.95 paper.

THE fifteen papers in this collection, all published previously in a variety of books and journals over the last twenty years, are printed here largely in their original form, but with the addition of a short general introduction to explain why they are all brought together into one volume. Each deals in some way with what Professor Holton terms the "themata" of science, that is the "unverifiable, unfalsifiable, and yet not-quite-arbitrary hypotheses" which are based not on empirical experiment and observation, nor on logical deduction, but simply on our general intuitions and beliefs about how we expect nature to behave.

The first three essays attempt to explain the concept of themata at length; the second essay concentrates on the metaphysical ideas underlying Kepler's description of planetary motions, and uses these as an illustration of how such ideas—in Kepler's work the belief that the Sun must be the centre of the Universe, and the belief in the underlying mathematical harmony of nature—form an essential part of the processes of scientific discovery. This is the only essay in which Professor Holton makes a lengthy excursion into early science; elsewhere his examples are drawn mainly from modern physics.

The second section, which comprises well over a third of the book, is devoted to relativity theory. The papers here, still very much concerned with the general question of underlying themata, cover the field of Professor Holton's most thorough historical research, and reflect his extensive study of the material in the Einstein Archive at Princeton Institute for Advanced Study. In particular the ninth essay shows, by extensive reference to source material, that Einstein received the inspiration for his relativity theory not so much from the famous Michelson-Morley interferometer experiments (as tradition would have it) as from the thematic influences from his contemporaries and from his own view of nature.

The next three essays are devoted to a discussion of how the themata of science (here referred to as the "non-rational substructure") are becoming

increasingly less acceptable as a facet of formal science. A distinction is drawn between "private science" and "public science"—the former the unrecorded progress of the individual towards a new theory, and the latter its presentation to the outside world, stripped of all description of the initial gropings of its author and set down tidily and rationally. The remaining two essays in the book deal with the role of science in education; Professor Holton asserts here that it is both essential and possible to give the man in the street, or at least the schoolboy at his desk, an adequate awareness of the present state of science, an awareness necessary to the health of our technologically based society. We must not only look outside science for some of the themata which govern scientific discovery, but must also allow science itself to operate in a reciprocal fashion upon our other fields of understanding. To achieve this, there must be a radical redesign of school and college curricula.

All the essays are fascinating and thought provoking (in his introduction Professor Holton writes "a chief aim of these essays is to raise new questions for research rather than merely to answer old ones"), and they are written for the most part in a language accessible to scientist and historian alike, despite the fact that many of the topics treated are by no means easy to present briefly and clearly to the uninformed reader. But there is some clumsiness in his exposition of the meaning of the term "themata" both in the introduction and in the first essay; his use of a three dimensional "space", with x , y and z directions representing respectively the empirical, logical and thematic components of scientific propositions seems to me both unnecessary and confusing. The concept of themata is not so complicated as to warrant such a laboured model for its explanation.

There are two difficulties inherent in an unedited collection of papers of this sort. First, there is inevitable overlapping between the papers, and here this is particularly so in the section on relativity theory; it is tempting to wish that the papers had been integrated into one coherent whole. Second, although the title of the book implies a common thread which runs through all the papers, many of them were not written with the primary or single purpose of describing the "thematic origins of scientific thought". Thus the twelfth

essay, *Models for Understanding the Growth of Research*, is more a study of the rapid increase in scientific information and investigation in recent decades and its general causes than a specific study of underlying themata currently guiding research.

The study of themata is by no means new in the history and philosophy of science (although this name for them is a little unfamiliar); and many of these papers were written so long ago that they have lost some of their original freshness—the author readily admits this. But for the scientist coming newly to the history of his subject, or for the layman seeking some insight into the nature of research, this collection of papers should prove enlightening, and convince him of the essential humanity of science.

LAURA TILLING

Disease distribution

Epidemiology of Neurologic and Sense Organ Disorders. By Leonard T. Kurland, John F. Kurtzke, and Irving D. Goldberg. Pp. xxx+436. (Harvard University: Cambridge; Oxford University: London, September 1973.) £7.

THIS monograph is one of a series of reviews of the epidemiology of various conditions published by the American Public Health Association. The series fills a long-standing need for material of this type. This particular book reviews morbidity and mortality data—a number of the others have confined themselves to the latter. The title, "Neurologic and Sense Organ Disorders", is slightly misleading because there is no reference to cerebrovascular accidents, cerebral tumours or cerebral infectious conditions. The volume is otherwise comprehensive.

The first chapter contains an introduction to epidemiology. This is inappropriate: it is too short to do justice to the subject and does not describe it well. Further chapters are devoted to convulsive disorders, Parkinsonism, multiple sclerosis, amyotrophic lateral sclerosis and other motor neurone disorders, muscular dystrophy and other myopathies, myasthenia gravis, Down's syndrome, congenital malformations of the nervous system and other specific neurological disorders, cerebral palsy, blindness and hearing disorders.

The first seven chapters, particularly those concerned with multiple sclerosis, amyotrophic lateral sclerosis and mus-

cular dystrophy, provide superb analyses of mortality and morbidity information as well as reviewing the aetiology of these conditions. The authors do not perhaps deal quite as well as they should with the problems of mortality certification in multiple sclerosis but they give one of the best descriptions I have read of the importance of epidemiological studies of migration in disentangling possible cause. The studies on epilepsy and other convulsive disorders give a most comprehensive review of the epidemiology of these conditions and demonstrate the value of mortality and morbidity statistics. There is also a reasonable analysis of diagnostic and other problems in this context.

The latter chapters are disappointing and highlight the difficulty of writing definitive works on such a wide variety of conditions. The chapters on Down's syndrome, congenital malformations and facial nerve palsy make no mention of possible aetiological factors and do not attempt to discuss the ways in which these conditions may arise. In the review of Down's syndrome, for example, nothing is said about the maternal age factor, the difference in aetiology between Down's syndrome in the offspring of young and old mothers, nor about the association between Down's syndrome and leukaemia.

With these reservations, the book is outstanding. WALTER W. HOLLAND

Sand surfaces

Atlas of Quartz Sand Surface Textures. By David Krinsley and John C. Doornkamp. (Cambridge Earth Science Series.) Pp. x+91. (Cambridge University: London, July 1973.) £6.50; \$19.50.

A METHOD of environmental analysis which depends upon descriptions of grain surface textures seen under the electron microscope from a wide variety of known environments has the inherent disadvantage that it requires a considerable accumulated experience and cannot yield results by a few simple measurements. Thus, the technique has largely been used by those prepared to invest a large amount of time, an investment which has seemed unlikely to be repaid by the results obtained. Krinsley and Doornkamp have now tried to make their experience available to those who might simply wish to use the technique as one of several means of analysis rather than embark upon an ambitious research programme. To do this, they have assembled a series of 122 excellent photographs which they claim to represent the principal associations of textures which, when taken together, could be used to characterise an environment.

Instead of the potential user having to undertake an exhaustive search of the

literature to construct for himself a pattern of variation, this is now done for him by the authors. The book also contains an introduction to the transmission and scanning electron microscopes, and their development and use in these studies, the preparation of samples; principles of interpretation; a particularly useful diagram showing the frequency of certain types of structures from a variety of environments; and a complete bibliography of the field.

It is clear that the book cannot be used directly as a key to environment, as it is the associations of textures which are important. The statistical analysis which this implies is not necessarily as easy as it may seem, for the number of clearly differentiated surface features which could be used in such an analysis are few. This leads to the general criticism that although analysis is based on descriptions of surface features, these are often vague and over-generalised, and often difficult to follow, such as the description on page 14 of mechanical V-shaped patterns. For example, although abundant reference is made to upturned plates, this term seems to cover a broad variety of forms which could usefully be differentiated.

For young research workers looking for rewarding topics, the book immediately suggests two. The possibility of quantified analysis; and the experimental reproduction of textures which might enable us to identify the processes at work and thus enable us better to specify the nature of the environment. The authors' own structural interpretations are sometimes unconvincing. But this lucid exposition in such a useful form will persuade many to use a technique which they may have previously regarded with awe or suspicion, and should find its way on to the bookshelves of most sedimentologists.

G. S. BOULTON

Fighting malaria

Malaria: The Design, Use and Mode of Action of Chemotherapeutic Agents. By R. M. Pinder. Pp. 336. (Scientifica: Bristol, September 1973.) £10.

THE preface of this book states that malaria remains a formidable global problem and that it is still the major killing disease of the tropics. One could not disagree with this statement even though the achievements of malaria eradication in many countries outside the truly tropical belt should not be underestimated. The partial failure of global malaria eradication is not due solely to the resistance of malaria parasites to drugs or to insecticide resistance of Anopheles vectors of the disease. This oversimplified explanation of a complex situation cannot be easily accepted by informed readers.

The book is composed of two unequal and rather unbalanced parts. Out of nine chapters six are devoted to chemotherapy of malaria; they comprise the testing and evaluation of antimalarials, a summary of the present status of various drugs, an outline of newer approaches to malaria chemotherapy, an account of miscellaneous compounds, including many experimental ones, a discussion of the mode of action of antimalarials and the review of the present status of drug resistance in malaria.

It is obvious that the author has had access to a formidable collection of reprints and index cards. And yet I missed in this part of the book an attempt at critical analysis of the laboriously collected information. It is as if anything that has been published were of equal importance just because it appeared in print. This outline of problems of chemotherapy of malaria supported by numerous references is, however, of some value although it cannot match the excellence and authoritative-ness of W. Peters's *Chemotherapy and Drug Resistance in Malaria* published in 1970.

The remaining three chapters of the book must have been written as an afterthought, perhaps to justify the all-embracing main title which overshadows the more appropriate subtitle. The chapter dealing with epidemiology of malaria gives a succinct account of the progress of malaria eradication and of its reverses; this is followed by a brief review of entomological and parasitological aspects. A chapter on malaria parasites includes the life cycle, the symptoms of clinical infection, and a notion of immunity. It is here that the author's nodding acquaintance with many aspects of malariology is evident. There are many statements that are either half true or quite wrong. Thus, neither pyrimethamine nor chloroquine has been "massively" used in common salt and the number of projects with medicated salt was small (pages 16-17). *P. falciparum* and *P. vivax* do not occur in higher apes in Africa (page 35). The population of tropical Africa is not infected *in toto* by quartan malaria (page 36). Sulphonamides are not causal prophylactic drugs (page 40).

The last chapter entitled "Malaria, the Way of the Dodo?" has also a quota of errors and infelicities (such as reference to the "wily plasmodium") that would be tedious to quote; however, the possibility of altering the man-biting habit of anopheline mosquito visualised by the author as a method of malaria eradication indicates his fleeting acquaintance with the discipline of malariology and with the realities of malaria control. To a question of for whom this book has been written I can give no satisfactory answer.

L. J. BRUCE-CHWATT

Language maths

Formal Languages. By Arto Salomaa. (ACM Monograph Series.) Pp. xiii+322. (Academic (Harcourt Brace Jovanovich): New York and London, May 1973.) \$19.

THIS excellent book is unique in its scope, detail and elegance. It covers almost the whole of formal language theory up to early 1972 in more or less detail, always with great clarity of style. The emphasis throughout is on pure mathematical theory—there is almost no discussion of applications. The author assumes a considerable mathematical stamina in his readers, and although the rigour of the text is considerably alleviated by many examples and side remarks, I would not recommend it as a first introduction to the field for most undergraduates.

The book is divided into three parts. The first (about a third of the book) is a very complete account of the classical theory of the basic hierarchy. The emphasis throughout is on the characterisation of language families by different means and the effects of various operations upon language families. Concepts of recursion theory are introduced informally at first but, in chapter 3, Smullyan's "rudimentary-predicate" characterisation is introduced as a descriptive device for type O languages. There is considerable new material, notably workspace theory. This section would form an excellent nucleus text for a short graduate course in formal language theory.

The second part—about half the book—is its real *raison d'être* for the professional reader. It amounts to a connected self-contained survey of all major and several minor developments in modern formal language theory. Topics covered—AFLs, controlled grammars of various kinds; Lindenmayer systems (very interesting); a very full discussion of context-free languages, including power series analysis, LR(k) and LL(k) grammars and ambiguity; and finally a sort of linguistic dustbin containing transformational, categorical, indexed, scattered context and probabilistic grammars. Some of these are treated rather briefly, but references to other fuller discussions are given. (Brief bibliographical and historical remarks follow each chapter.) The scope of this section, the detail and rigour of the proofs, and the pervading sense of mathematical elegance, make these chapters a unique reference source for the field. The author gives many fascinating side remarks and illustrative examples.

The third part seems like an appendage. It consists of a fairly standard account of decidability and a nice introduction to complexity theory. The former would have been better placed

nearer the beginning of the book: the latter should either have been omitted altogether, or should have been expanded to include (for example) a discussion of polynomial-time results for nondeterministic recognisers. At present it is an uneasy compromise.

The author has tried to give a complete account of a large subject. One can question whether it was sensible to make the attempt at this time, when the field is expanding so rapidly. Perhaps the publishers could leave a few blank pages in the next edition so that readers could jot down new results as they appear.

The book is technically well produced. I could find no significant misprints.

PATRICK J. HAYES

Brain damage

The Working Brain: An Introduction to Neuropsychology. By A. R. Luria. Translated by Basil Haigh. Pp. 398. (Allen Lane: London; Penguin: Harmondsworth, April 1973.) £2.50; Penguin £1.25.

ONE of the main problems with physiological psychology or neuropsychology is the crudity of its techniques relative to the complexity and subtlety exhibited by its subject matter, the brain. In this book one of the world's most eminent neuropsychologists surveys his vast work on the psychological effects of what is perhaps the crudest technique, brain damage. His purpose is to substantiate the claim that the resulting dysfunctions can tell one much about the functional structure underlying the cognitive and perceptual properties of the human mind and the mechanisms by which they are manifest in overt behaviour.

In the opening part of the book, Luria discusses lesion methodology and rejects the simple 'localisation of function' procedure in which the dysfunction is described as the loss of a certain ability; a process to mediate that ability is specified, and finally this process is localised in the particular brain area damaged. It is clear that in a complex mechanism such as the brain there is not necessarily any simple mapping between the domains of functional and anatomical organisation. The outcome of brain damage, defined by anatomical criteria, could well be behavioural disturbance which does not reflect in any meaningful way the structure of the processes mediating normal functioning. Luria's answer to this problem is that a "detailed psychological analysis of the structure of the disturbance and the elucidation of the immediate causes of the collapse of the functional system" is necessary. But here is the paradox. If an adequate psychological theory of a given ability is a necessary prerequisite of a "detailed qualification of the

symptom", what additional information about the psychological organisation can the study of brain damage give us? The question is whether the rest of the book provides an adequate answer.

Luria divides the brain into three principal functional units: the reticular activating system and the limbic system for regulating the arousal of the cortex and controlling emotional and motivational processes, the primary and secondary projection areas and nonspecific areas of the posterior cortex for processing and storage of sensory information, and finally the motor and premotor areas and frontal lobes for programming, regulation, and verification of activity. In the second part, the author summarises a large body of evidence, mainly from his own or associated sources, on the effects of brain damage in various cortical areas. This material is then recapitulated in the third part and organised in terms of psychological processes rather than anatomical criteria.

Although a summary of Luria's extensive work must be of value, the relatively naive reader has difficulty in evaluating the adequacy with which the dysfunctions are presented. Most of the descriptions are supported solely by clinical evidence and rarely are control data given for comparison. This difficulty is compounded by the appalling and often incomprehensible presentation of the figures; graphs have unlabelled axes and examples have unexplained legends. The theoretical versus descriptive status of the terms in which a syndrome is presented is often ambiguous, and I get the impression that Luria, in spite of his caution against 'the localisation of function' procedure, often reifies deficits. Luria's theoretical position is eclectic and he draws on many sources in specifying the psychological processes he thinks underlie the capacities of the mind. But the general theoretical mechanisms are presented in such an unspecified form that it is very difficult to decide whether brain damage really tells us anything new about psychological processes. A conclusion, which could find supportive evidence in this book, is that psychological theory is still too primitive to allow the proper use of the patterns of behavioural breakdown induced by brain damage.

ANTHONY DICKINSON

New book?

Quantum Statistical Properties of Radiation. By W. H. Louisell. Pp. xiv+528. (Wiley: New York and London, July 1973.) £14.

THIS book is really a second edition of the author's 1964 volume *Radiation and Noise in Quantum Electronics* published by McGraw-Hill. Why he has changed his publisher and title I cannot

imagine. Apart from a few small changes, the first 330 pages are word for word copies of the earlier work (the use of a pair of scissors to rearrange some of the sections should not confuse the comparison). The claim on the dust jacket that this material has not been available previously in book form took me by surprise. Let me suppose, however, that the bookseller has been persuaded to divide the spine at page 330 and has accepted back also seven appendices; this leaves £4.25 worth of new material at a *pro rata* rate per page. The new material consists of four chapters, two on the quantum theory of damping and two on the theory of the laser.

The author's approach to these matters is based on the idea, explicitly stated on the dust jacket, that *c*-number equations are more easily solved than quantum equations. The earlier book was devoted to the elaboration of the technique of the Wigner distribution function and related functions for converting quantum mechanical equations into apparently classical equations. The idea is not at all proved (or even discussed further in the text) and, in fact, a quick thumb through these chapters would indicate to a trained mathematical physicist that the opposite might be the case. This is particularly true of quantum optics, where spontaneous emission, which is the key to quantum noise, is trivially easy to identify in the quantum equations but is deeply buried (finally even to disappear in the classical limit) in the equations of classical form.

There is no accounting for taste, however, and ignoring the strictures above, Professor Louisell has done a very thorough job within his brief. The earlier chapter 3 is augmented by five new "ordering" theorems and the detailed mechanisms of the damping of a system by interaction with a reservoir are worked through in the first of the chapters on quantum damping. In the second of these chapters, damping is studied from the point of view of Langevin forces which are derived consistently with quantum commutation requirements. No contact, unfortunately, is made with modern Langevin statistical methods such as those of Kubo and Mori. Finally, the theory is expressed in *c*-number form by use of appropriate ordered distributions. This is material familiar to those who have studied the methods of Lax and his colleagues over the years and is convenient to have in a form other than the original papers or summer-school notes.

Of the two chapters on the laser, the first discusses the semiclassical theory after Lamb, and the second statistical properties following the Fokker-Planck treatment due independently to the schools of Lax and of Haken. No great depth is achieved and I could trace no

mention in the whole book of anything so mundane as an experiment.

At £14 this work will not appeal to the general optical physicist. The theoretical specialist, who will have the earlier volume, will be loath to spend this sum for so little new material.

E. R. PIKE

Atmospheric dynamics

Atmospheric Energetics. By Jacques Van Mieghem. Pp. ix+306. (Clarendon: Oxford; Oxford University: London, April 1973.) £7.50.

THIS treatise is based on the author's lectures on atmospheric dynamics at the University of Brussels and at the seminar of the Department of Aerology of the Royal Meteorological Institute of Belgium. Part 1, comprising the first seven chapters, is devoted to the basic energy equations for laminar and turbulent flow. There then follow chapters on the energetics of small-scale forced and free convection.

The main emphasis of the book lies, however, in the treatment of the energetics of large-scale atmospheric systems and the author develops this topic extensively, reflecting his own considerable original contributions in this field. Chapter 11 considers the generation of eddy kinetic energy by conversion of potential energy and its transfer to the mean zonal motion by the long waves in the westerlies; chapter 12 deals with the basic energetics of the general circulation with particular emphasis on the role of heat sources and sinks, and chapter 13 the relationship between internal and potential energy in the process of generation of kinetic energy on the synoptic scale. Chapter 14 (the longest in the book) is devoted mainly to an extensive development of the concept of available potential energy. Chapter 15 discusses the results of computations of the energy partitions and transformations between the mean zonal flow and the eddies using atmospheric data. The last three chapters are concerned with theoretical results on the transfer of wave energy, the role of ageostrophic motion in the energetics of the general circulation and the consistent formulation of simplified mathematical models of the atmosphere satisfying particular conservation laws.

Little comment is required concerning part 1 of this book and the first three chapters of part 2. The presentation is clear and the whole very carefully written. The chapters on free and forced small-scale convection are particularly well presented and include good examples of observed data illustrating the theory. Chapters 11 to 15, however, devoted to the energetics of the general circulation and comprising nearly half the book, although containing a wealth of mathematical theory,

again very carefully and rigorously presented, somehow seem to lack a well defined sense of direction. The mathematical development is in places long and even tedious with little motivation (or apology!) supplied to the reader—for example in the section devoted to the first and second time derivatives of the total potential energy. Some improvement could be achieved by a more informative choice of chapter and section headings—what, for instance, is the reader supposed to infer regarding the distinction between the material in chapter 11 entitled "The Energetics of Large-scale Eddies" from that in chapter 14 entitled "The Energetics of Quasi-static Motion Systems"? The latter title seems in any case to be a self-contradiction! But these are essentially criticisms of presentation; the material in these chapters, like that in the rest of the book, conforms to the highest standards of scientific rigour.

The last three chapters are each more or less self-contained and relatively easy to follow.

There is little doubt that this scholarly treatise will rapidly become established as a standard reference book for research workers in dynamical meteorology. It probes to a more fundamental level than any other existing work into the consequences of applying the principle of energy conservation to the atmosphere. In doing so it leaves the reader with a deeper appreciation of how the atmosphere works. Perhaps, at the same time, it will leave some wondering whether there is very much more left to be discovered by attempting to refine still further mathematical methods for applying this particular principle to atmospheric dynamics without, at least, first thoroughly examining the consequences of applying the theory as it now stands.

R. P. PEARCE

Inside stars

Physics of Stellar Interiors. By Thomas L. Swihart. (Astronomy and Astrophysics Series.) Pp. x+119. (Pachart: Tucson, 1972.)

THE author of this book is well known for a textbook published five years ago in which he managed to give a remarkably concise and equally clear introduction into the fundamental concepts of astrophysics and stellar astronomy for the benefit of physics students. Three years later he wrote a short text which deals in less than eighty pages with the basic physics of stellar atmospheres and succeeds well in giving to the more advanced physics student a good general idea of the various aspects of this all important subject.

The success of these earlier books and their proven usefulness in the teaching

of astrophysics has made many people look forward to a text on stellar structure from the pen of Professor Swihart. Such a text has now appeared in the form of a companion to his earlier *Basic Physics of Stellar Atmospheres*, and as an introduction into the field of stellar structure the new book can be quite as strongly recommended as Professor Swihart's earlier writings. This book is a short introductory text, but one which contains all the essential points. There are discussions of conditions of gas in thermodynamic equilibrium, of polytropes, of energy generation and transport, of radiative transfer and opacity. There is a final chapter devoted to a discussion of theoretical stellar models and to their evolutionary changes as the result of changes in their chemical composition. Present problems and discrepancies are as clearly stated as the remarkable overall agreement between theory and observation which exists in this field.

As in his earlier book the author has again found room in an appendix for a dozen problems whose solution will test a student's understanding of the text. The only change which I would like to suggest for the next edition of this most useful book is the adoption of the international system of units.

H. A. BRÜCK

Chemicals in soil

Organic Chemicals in the Soil Environment. Edited by Cleve A. I. Goring and John W. Hamaker. (Books on Soils and the Environment Series.) Vol. 1, pp. xii+440; vol. 2, pp. xii+441-968. (Dekker: New York, August and September 1972.) Vol. 1, \$24.50; vol. 2, \$26.50.

CHEMICAL pollution of the environment is highly emotive, but solid facts are often lacking, so the publication of this tremendous work is certainly timely. Most people understand that the soils which mantle the Earth are a basic resource to be carefully husbanded in order to increase the world output of food and other raw materials. To achieve this end man has come to depend more and more on the application of fertilisers through foliar sprays or, more usually, through the roots by way of the soil; on optimising the water supply through the soil; and on minimising biological competition from weeds, pests and diseases through foliar or soil applications of crop protection chemicals.

This work comprises thirteen chapters grouped in four parts dealing respectively in vol. 1 with "The Soil Environment" (one chapter) and "Physiochemical Relationships of Organic Chemicals in Soil" (five chapters); and in vol. 2 with "Action and Fate of Organic Chemicals in Soil" (five chapters) and "Environmental Effects of Organic

Chemicals" (two chapters).

The editors head agricultural research at the Ag-Organics Department of the Dow Chemical Company in California. Both are men of distinguished research and organising ability; they have themselves contributed four chapters and enlisted the able help of colleagues, Drs Brandt and Meikle, to write two others, so that about half the total work emanates from the Dow Company. The remaining authors are all highly competent researchers and writers, including Dr Edwards from Rothamsted Experimental Station and Dr Thomas of Birmingham University; the net result is a well balanced text.

Chapter 1 by J. L. Ahrlichs of Purdue University is a comprehensive introductory account of macrocharacters of soils as they occur naturally and of the microcharacters of the soil solid, liquid, gaseous and living phases including some references to enzyme activity: some readers may be puzzled by the USDA "7th approximation" alternative names for soil horizons.

Chapter 2 by J. W. Hamaker and J. M. Thomson is a very detailed and valuable treatment of surface absorption principles. Chapter 3 by R. W. Meikle deals mainly with the biochemical mechanisms of decomposition, in soil systems, of the synthetic organic chemicals used for crop protection and includes a discussion of the "permease" concepts of active transport across cell membranes which those not familiar with these ideas will appreciate. Chapters 4 and 5 by Hamaker are concerned respectively with detailed reaction kinetics in relation to conditions in the soil environment and with diffusion and volatilisation in the gaseous phase. Chapter 6 by Professor Letey and J. Oddson continues the theme of movement in the soil system, chiefly through mass flow in aqueous solution.

Chapters 7, 8 and 9 are respectively excellent reviews of herbicides and plant growth regulators (R. P. Upchurch), insecticides (C. A. Edwards), and fumigants, fungicides and nematocides (C. A. Goring). Then comes a change from the dominant crop protection materials to a consideration by J. D. Hauck in chapter 10 of synthetic slow release fertilisers and fertiliser amendments, and in chapter 11 of soil physical property modifiers by G. H. Brandt.

The final part is devoted to a chapter by Professor Martin on side effects of organic chemicals on soil properties and plant growth, and one on Goring's viewpoint of the impact of agricultural chemicals on the environment with some valuable data on input levels, accumulations and toxicity levels.

Every chapter is well written and illustrated, with very few typographical errors. Each concludes with numerous

references and at the end of vol. 2 there is a very useful list of the full chemical names against each of the common or temporary names quoted, followed by comprehensive author and subject indices. Though the books are costly for private pockets they are highly recommended to all who have a professional interest in the subject and for library acquisition. The authors and editors are to be congratulated on bringing together in comprehensive, readable form a mass of detailed information, much of which has hitherto been inaccessible to the average inquirer. Furthermore, it is infused with a good many stimulating ideas and comments.

J. TINSLEY

Crystal physics

Tensors and Group Theory for the Physical Properties of Crystals. By W. A. Wooster. Pp. x+344. (Clarendon: Oxford; Oxford University: London, August 1973.) £7.

THIS book may be regarded as an expanded and modernised version of Dr Wooster's well known *Crystal Physics* which was first published in 1938. Its principal objective is "to enable students to grasp the essential techniques of dealing with tensors and group theory as they are applied to crystals". Numerous support exercises are interwoven with the text to which detailed answers are provided. The author's mastery of crystal symmetry and crystal physics comes through on almost every page, but whether he has achieved his stated objective remains open to question.

Tensors and groups are introduced in a piecemeal fashion without being built up into any coherent body of theory. This approach might well give the student deeper insight into the meaning of the symbolism, as Wooster points out in his introduction, but a heavy price must be paid. The theory of the crystallographic groups cannot really be understood without reference to the basic finite groups, including particularly the cyclic, dihedral and tetrahedral groups. Any attempt to bypass these leads inevitably to lacklustre multiplication tables and unduly complicates the determination of the conjugation classes. It also precludes the recognition of useful isomorphisms between apparently different groups. Similarly the discussion of polar and axial vectors suffers from an inadequate mathematical framework. It is no paradox to suggest that a dash more of mathematics could have reduced the length of this book and improved its clarity. But, despite these criticisms, it makes a refreshing change from the over-mathematical texts which hardly make any contact with physical ideas, and it contains a tremendous amount of valuable information. M. A. JASWON

Enhancing immunity

Immunopotential (Ciba foundation Symposium 18, New Series.) Pp. ix+335. (Elsevier/Excerpta Medica/North-Holland, Associated Scientific Publishers: Amsterdam, London and New York, 1973). Dfl. 47.50; \$18.30.

THIS symposium deals with the effects of substances (adjuvants) which can stimulate the immune response to unrelated antigens. This topic is of obvious interest as far as control of the immune response is concerned, but becomes of major importance because of its clinical significance. Adjuvants can help in cancer therapy, and the proceedings summarise the situation in this area, and suggest mechanisms whereby adjuvants may be acting.

Convincing evidence is presented of the efficacy of immunotherapy for cancer treatment in laboratory animals and human disease. A rationale for the use of adjuvants in some human leukaemias is clearly advanced by Mathé. If chemotherapy is first used to reduce the tumour mass, immunotherapy involving the injection of BCG and killed tumour cells can be strikingly successful in prolonging remission of the disease.

It seems unlikely that the agents useful in cancer therapy act on the immune system, mainly because they are also powerful adjuvants for many well characterised immune responses. The effects of adjuvants on these responses is also dealt with. Some participants suggest that adjuvants may potentiate the trapping of lymphocytes in lymph nodes or spleen and improve cell interactions. Others favour the view that adjuvants directly stimulate cells involved in immune responses. In this regard the B lymphocyte, T lymphocyte, macrophage triangle is dominant, and effects on all three cell types are proposed. Perhaps one can generalise to say that macrophages certainly seem to be strongly involved in adjuvant action, and that different adjuvants can have effects biased towards B or T lymphocyte responses. Some can even potentiate one type of immune response and suppress another.

As the proceedings make clear, what is needed is an understanding of the control mechanisms involved, and hopefully the discovery of methods of selectively stimulating or suppressing the different types of immune response.

Ciba Symposium 18 should be of great interest to those studying the immune response or cancer therapy. Immunopotential is a confusing subject, and the presentation of the 1973 state of play is of great value; the discussions of the participants are particularly useful. It is clear that there is work to be done, and anyone reading

the volume should find a few ideas to begin with.

A. F. WILLIAMS

Creative stimulus

Population Growth: Anthropological Implications. Edited by Brian Spooner. Pp. xxvii + 425. (MIT: Cambridge Massachusetts and London, 1973.) \$15; £6.75.

THIS collection of papers considers various anthropological problems in the light of the population theories of Ester Boserup¹. She considers that human population growth acts as an independent variable in the development of culture and society. Population growth is not inexorably limited by the level of resources through the agency of 'misery and vice' but instead stimulates invention to generate new resources or new techniques for exploiting old resources.

There are seventeen papers in all. The first six are concerned with archaeological evidence. A mass of archaeological data is presented for Mesoamerica, Mesopotamia and Egypt. Sometimes this is accompanied by population density estimates, sometimes not. In most of these papers the relationship of population change, resource exploitation and economic theory is treated very much as a postscript.

Bronson's paper "Farm Labour and the Evolution of Food Production" is one of the few where theory is discussed at length. According to Boserup, farmers will always prefer easy extensive cultivation (like shifting cultivation) until they are forced to intensify methods by population pressure. Furthermore, farmers will revert to shifting cultivation if population size falls. But where population is known to have fallen, as in Europe after Rome's fall, no such agricultural change is seen. Bronson also complains that the more elaborate stages of agriculture should not necessarily be regarded as being more 'adaptive' than the simpler ones, especially when the latter are still represented in present-day economies. If Boserup were correct, shifting agriculture would be extinct, whereas it is still widely practiced in areas which could support more intensive systems.

Most of the rest of the book deals with present-day populations from the viewpoint of social anthropology. One paper considers the importance of religious organisation and suzerainty in achieving political centralisation. Another describes conditions in Tibet which support Boserup's theory through its obverse: despite a superabundance of resources a population declines for non-economic reasons and migration

takes place into the area of its most extensive deployment; high altitude nomadic pastoralism. One of the most interesting papers presents calculations to show how the problems of carrying children force the nomadic Kung bushmen to prolong the interval between births; a type of problem not dealt with in Boserup's economic formulations.

As a discussion of Boserup's theory in relation to anthropology this book is a disappointment, and the title is certainly misleading. The archaeological data are doubtless interesting in themselves and some of the population estimates may be useful. But demographic data from archaeological and ancient literary sources are unreliable and may always remain so. Associations may be established between past population growth and technical change, but Boserup's theory seems to require population change to occur first and, of course, for the postulated mechanism to be responsible. This would be difficult enough to establish in a modern population and it is not clear how the necessarily less detailed information from archaeology could hope to validate such a theory.

But most of the papers on modern populations are also unable to demonstrate the importance of the demographic factor, partly through their lamentable lack of numerical information. In many cases no demographic data are quoted, population is rarely mentioned and resources are not evaluated.

It is very surprising that no reference is made to historical European populations whose demography and economics are much better known than those of ancient Egypt or Mesopotamia. Exogenous technical innovations, which have had profound demographic consequences, might also have been germane to these discussions. But the introduction of the potato to Europe, or of European sanitary and medical knowledge to the Third World, are nowhere mentioned.

It seems that many archaeologists and anthropologists need to know more demography. The difficulty of establishing a trustworthy case for population change does not seem to be appreciated, the impossibility of sustained population growth or decline for more than a short time is not realised, and the views of Malthus and other theorists (often thought to be in opposition to Boserup) are widely misunderstood by the contributors to this book.

D. A. COLEMAN

¹ Boserup, E., *The Conditions of Agricultural Growth: The economics of Agricultural change under Population Pressure* (Aldine, Chicago, 1965)

Desert landforms

Geomorphology in Deserts. By Ronald U. Cooke and Andrew Warren. Pp. 374. 80 plates. (Batsford: London, August 1973.) £5.50.

LANDFORMS in deserts, unobscured by vegetation and constituting the scenery, are remarkable for their abruptness and orderliness. Until recently there were few books in English concerned mainly with desert geomorphology. *Deserts of the World* by W. G. McGinnies *et al.* (Office of Arid Lands, University of Arizona, 1968) contains only one chapter on geomorphology; K. W. Glennie's *Desert Sedimentary Environments* (1970) is more specialised, but the volume under review rarely overlaps it.

The field experience of the two authors has been complementary. Cooke has published on pediments, gullies and desert pavements in North and South America. Warren has worked on soils and dunes in Pakistan and the Sahara.

Their book is in four parts. In the first the diversity of desert landforms is emphasised and different approaches to their study are assessed. The concepts of W. M. Davis are treated rather unsympathetically but the wonder is that his arid cycle has been treated so seriously; he obviously regarded it as a sighting shot. The authors themselves adopt a more fashionable systems approach, one which I think is not ideally suited to a subject in which discontinuity of operations is so marked.

Most of the world's deserts, even the southern Sahara, were not truly arid as recently as 5,000 to 15,000 years ago and before that their climates, at least through the Quaternary, fluctuated markedly. Such instability seems to me to present difficulties for a systems approach to the study of the resultant landforms. I am not convinced that the return period concept releases geomorphologists from the necessity of concerning themselves with paleoclimatology; the two must go together.

The soil surface system is the subject of part 2. It brings together very usefully a great deal of recent literature on desert soils. Part 3 deals with integrated patterns of change on slopes and in channels, and with features such as pediments, fans and playas. There should be more photographs illustrating these features. Pediments remain enigmatic; as with so many forms that attract attention, their vital statistics characterise without necessarily revealing their underlying nature.

In part 4, "Aeolian Systems," a couple of equations have somehow been missed out and there are a number of minor slips, but the section on dune patterns is very stimulating. It draws on the work of the late Ian Wilson and I hope

that Warren and others will pursue the subject further with the cooperation of aerodynamicists.

A. T. GROVE

All immunology

Handbook of Experimental Immunology. Edited by D. M. Weir. Second edition. Pp. 350. (Blackwell Scientific: Oxford and London, 1973.) £22.50.

THE second edition of the handbook weighs 3,000 g, an indication both of the editor's assiduity and of the growth of immunology (37% increase in weight over the previous edition!). The utility of this monster will be revealed with time and the passage of many toil-stained fingers but even now deficiencies are clear.

The three sections of the book are labelled "Immunochemistry", "Cellular Immunology" and "Applied Immunology." Individual chapters are written in the main by people acknowledged by their peers as experts in particular fields. But it is already nearly two years out of date which in cellular immunology would by many be judged a crime. Even if it were up to date it is not comprehensive and this is particularly true of the applied immunology section. Hardly any of the current attempts to apply cellular immunological methods, experimentally, in clinical contexts or in studies of experimental infection are documented in the handbook; there is very little on application of the methods of rosette-forming cells, anti-theta analysis, cell electrophoresis, velocity or density sedimentation of lymphocytes and general surface antigens of lymphoid cells. Many methods are given in such detail as to duplicate other parts of the book. The many recipes for PBS phosphate-buffered saline are a case in point. Judicious editing might have avoided quite so much overlap and thereby reduced the expense. The index is the only form of cross-referencing between chapters which otherwise, and perhaps inevitably, seem to have been written in isolation.

On the good side are the very many chapters which do beautifully exemplify the contemporary immunological scene. The illustrations are good and clear almost throughout the book. There is a wealth of the folk-lore of immunological method honestly set down. The handbook is an heroic effort but its high price and the speed of change in immunology combine to make it as far as its cellular immunological parts are concerned a white elephant.

A. J. S. DAVIES

Black holes expand minds

Black Holes: The End of the Universe? by John Taylor. Pp. 174. (Souvenir: London, October 1973.) £2.50.

It is difficult to see this book as other than an attempt to pre-empt future science fiction writers by attaching a scare story of death and destruction to every aspect of black holes.

The book is glued together with long tedious passages discussing the significance of black holes to mankind and the author uses the first forty pages to make general remarks about the state of the world, religion, precognition, astrology and so on. He announces that America has cut oil supplied to airlines, that Heitsi-ebib, a god of the Hottentots, died several times, that when Huxley and Wilberforce argued on evolution Lady Brewster fainted, and many other gratuitous scraps.

Then black holes are introduced. The chapter starts "None of us wants to die, but death is with us whether we like it or not; sadly we have not yet discovered how to avoid it". For the next hundred pages the author wanders through various properties of black holes with an unerring eye for the spine chilling. "The pulverized mess that was [the astronaut's] remains would be crushed ever smaller". "The bones and muscles of his body will be elongated beyond breaking point". This is as unhelpful as would be a ceaseless emphasis in a book on plate tectonics, on the horrid death to be expected if one stayed on an oceanic plate at a subduction zone.

It would be difficult for a layman to appreciate whether the chapter on extracting energy is anything other than pure tongue-in-cheek. It would be equally difficult for him to grasp some of the concepts which are introduced in such an off-hand way—in phrases such as "Time passes very, very slowly for the first few billionths of a second (of the universe)" or "Light possesses energy and so has mass". Professor Taylor declares it "certain" that there is life elsewhere in the Universe and goes on to guess what it is like. The relevance of this to black holes is re-revealed in breathtaking manner when the biblical legends of Satan are interpreted as God casting Satan into a black hole (the "fiery furnace"). The Devil's horns "could indicate antennae" confirming that he came from distant parts of the Galaxy.

The author says in the concluding paragraph of the book that the black hole Universe affords "great opportunities to expand our vision of the world". Just so.

DAVID DAVIES

matters arising

Rapid fluctuations of large volume astronomical sources

SIR,—The argument that the characteristic fluctuation time of an astronomical source implies that the source be smaller than a characteristic dimension is often quoted without adequate discretion, particularly concerning QSOs¹ and pulsars. That argument is actually valid only for incoherent generation of radiation and since non-thermal radiation mechanisms are strongly suspected the validity of the argument must also be suspected.

As a simple counter-example I take the case of a rapidly fluctuating star situated in a large absorbing cloud. For an external observer the star appears to have unaltered dimensions and fluctuations, but lower brightness temperature, compared to if the cloud were absent. Now if the cloud were to have negative

absorption (maser amplification) the external observer would still see the same dimensions and fluctuations, but higher brightness temperature, again compared to if the cloud were absent. The observer would not see the cloud itself even though the power generation could occur throughout the full volume of the cloud.

Such a mechanism could clearly alleviate the power density dilemma of quasars even at cosmologic distances. QSOs would be able to fluctuate rapidly and also appear small even though the power generating volume were large.

It turns out that such an amplifying cloud could also alleviate the second principal dilemma for cosmological QSOs; their apparent connections with peculiar local galaxies. Rozyczka² has mentioned three possible explanations for those connections. They may be: (1) real; (2) by chance; or (3) caused by the amplification of radiation in the vicinity of the peculiar local galaxies. Large

amplifying clouds, this time surrounding galaxies, again might conciliate puzzling observations.

My purpose here is not to advocate amplifying clouds, for they raise problems as serious as those they solve. The source of energy and the required pumping and amplification mechanisms would pose enormous difficulties. (The energy source is actually a serious problem for almost any explanation.) The purpose is rather to provide a reminder that thorough and meticulous logic must be exercised during deductions if the conclusions are to be valid.

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¹ Burbidge, G. R., *Nature phys. Sci.*, **246**, 17 (1973).

² Rozyczka, M., *Acta Astr.*, **23**, 233 (1973).

Announcements

Appointments

B. S. Hartley of the Medical Research Council Laboratory of Molecular Biology at Cambridge has been appointed to the chair of biochemistry at Imperial College, London.

G. E. Eden has been appointed to take charge of the Water Pollution Research Laboratory, Stevenage.

W. G. Crewther has been appointed chief for the CSIRO Division of Protein Chemistry.

International Meetings

February 12, **High Performance Liquid Chromatography: A forum for the Exchange of Experience with Apparatus** (The Society for Analytical Chemistry, Analytical Division, Chemical Society, 9/10 Saville Row, London W1X 1AF)

February 13, **Fundamentals of Remote Sensing** (General Secretary of the Remote Sensing Society, Dr W. G. Collins, Reader in Remote Sensing, Remote Sensing Unit, University of Aston,

Gosta Green, Birmingham, B47ET)

February 13–15, **Biological effects of Electromagnetic Radiation** (Public Relations, The New York Academy of Sciences, 2 East 63rd Street, New York 10021)

February 19, **High Performance Liquid Chromatography** (The Society for Analytical Chemistry, Analytical Division, Chemical Society, 9/10 Saville Row, London W1X 1AF)

February 20, **Automatic Turbidimetry** (The Society for Analytical Chemistry, Analytical Division, Chemical Society, 9/10 Saville Row, London W1X 1AF)

February 20, **The Rational Control of Sterile Areas** (The Society for Analytical Chemistry, Analytical Division, Chemical Society, 9/10 Saville Row, London W1X 1AF)

February 20–21, **The origin of Cosmic Radiation** (The Executive Secretary, The Royal Society, 6, Carlton House Terrace, London SW1Y 5AG)

February 27, **Secondary Steelmaking** (Meetings Secretary, Institution of Metallurgists, Northway House, Whetstone, London N20 9LW)

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Greater London Council Scientific Branch. Annual Report of the Scientific Adviser 1972. Pp. 136. (London: Greater London Council, 1973.) £4.

British and Continental Progress in Water Pollution Control. (Sixth Public Health Engineering Conference, held in the Department of Civil Engineering, Loughborough University of Technology, January 1973.) Edited by John Pickford. Pp. 86. (Loughborough: John Pickford, Department of Civil Engineering, University of Technology, 1974.) £3.

The Astronomer as Natural Philosopher. By Professor A. H. Cook. (An Inaugural Lecture.) Pp. 36. (London: Cambridge University Press, 1973.) 45p net; \$1.75.

ARC Index of Agriculture and Food Research. Pp. viii + 132. (London: Agricultural Research Council, 1973.) £1.

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